A study of the colonial organisation of two species of arborescent cellularine Bryozoa.

Fairall, Victor Roger

A study of the colonial organisation of two species of arborescent cellularine Bryozoa

by

Victor Roger Fairall, B.Sc.

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789 East Eisenhower Parkway
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Ann Arbor, MI 48106 – 1346

DECLARATION

Declaration in accordance with Regulations 11 and 18 for the degree of Philosophiae Doctor:

I declare that the work submitted in this thesis under the title "A study of the colonial organisation of two species of arborescent cellularine Bryozoa" is the result of original research. It has not already been accepted in substance for any degree, nor is it currently submitted in candidature for any degree. All authors and works to which reference has been made are fully acknowledged. I hereby give consent for my thesis, if accepted, to be available for photocopying and for inter-library loan and for the title to be made available to outside organisations.

Candidate..

Supervisor.

Date.....

To Liane, my wife.

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SUMMARY

The central aim of the study was to describe the spatial arrangement of the zooids within a colony of *Scrupocellaria reptans,* in sufficient detail to reveal previously undescribed characteristics.

A rudimentary population study used monthly sampling to ascertain basic biological characteristics of the species. These included settlement period, colony size changes over time, and longevity. Settlement period was June to August; colonies were annual, but suffered extensive partial mortality mid-winter.

The inadvertent collection of *Tricellaria inopinata,* a species new to Britain, by a colleague, necessitated an investigation into its confused taxonomy, via the literature and historical and recent material. The species was differentiated from two similar species.

A detailed study was made of the spatial arrangement of autozooids and polymorphic heterozooids within a colony of *S. reptans.* The arrangement of autozooids could reveal details of colony structure, whilst that of polymorphs, not easily 'understood', could suggest new lines of enquiry. The methodology involved 'mapping' the spatial arrangement of zooids within a colony, in respect of a number of parameters, in such a way that they could be investigated singly or in any combination. A similar study was made in respect of *T. inopinata.* Did any new characteristics of *S. reptans* occur more widely?

Colonies of both species had a definite structure and form. The structure, which involved a small number of long sequences of short 'internodes', from each of which laterally limited 'aggregations' of short sequences, of generally longer 'internodes', developed, essentially the same in both species. The form was slightly different. Polymorphs occurred constantly, predictably or unpredictably. The spatial arrangement of the latter was very complex, probably involved an intrinsically spatial element, positive and negative associations between polymorphs, and their level of occurrence. There were numerous asymmetries of occurrence, many unexpected, and some answers, but many unanswered questions.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 2

Chapter 3

Chapter 5

Chapter 6

LIST OF FIGURES

LIST OF APPENDICES

The appendices below are all on Compact Disc

Appendix 'F', Raw data, re the arrangement of autozooids, internodes, polymorphs and ovicells, within certain internodes developing from the longest 'stem sequence', in the colony of *Scrupocellaria reptans* from Musselwick, studied in detail (Chapter 5).

Appendix 'G', Raw data, re the arrangement of autozooids, internodes, polymorphs and ovicells, developing from the long 'stem sequence' originating from internode E15, in the colony of *Scrupocellaria reptans*, from Musselwick, studied in detail (Chapter 5).

Appendix 'H', Raw data, re the arrangement of autozooids, internodes, polymorphs and ovicells, developing from a long 'stem sequence', from a colony of *Scrupocellaria reptans*, from Swanage (Chapter 5).

Appendix 'J', Raw data, re the arrangement of autozooids and internodes, within one colony of *Tricellaria inopinata,* studied in detail (Chapter 6).

Appendix 'L', Raw data, re the arrangement of autozooids, internodes, polymorphs and ovicells, developing from a second long 'stem sequence' of *Tricellaria inopinata*, (originating in an unknown generation), from Poole Harbour (Chapter 6).

Appendix 'M', Raw data, re the arrangement of autozooids, internodes, polymorphs and ovicells, developing from a long 'stem sequence' of *Tricellaria inopinata* (originating in generation 'H') from Poole Harbour (Chapter 6).

CHAPTER 1 INTRODUCTION

1.1 ORGANISATION OF THESIS

Before introducing the central focus of this thesis and discussing the broad field that underlies it, it is necessary to outline its overall arrangement and the contents of the chapters within it.

Chapter 1 provides a general introduction to the subject of colonial life and its taxonomic concentration in the eusocial insects and colonial marine invertebrates. The similarities and differences between the two groups are briefly considered, with particular emphasis on polymorphism, how it arises, and the extent of its occurrence. This is followed by a general introduction to the Bryozoa, their general biology, structure, classification and growth forms. Particular emphasis is placed on their colonial nature, variations in the level of colonial integration, and the role of polymorphic individuals within this.

Chapter 2 is a very preliminary population study that aimed only to establish certain basic biological characteristics of the species central to the study, *Scrupocellaria reptans*. I felt I should know when settlement occurred, how colonies grew, how large they became, and how long they lived; before conducting a detailed study of the way zooids were spatially arranged within a colony.

Chapter 3 is concerned with the description and taxonomic identity of a species of cellularine Bryozoa, which was collected by a colleague as *S. reptans*, but proved to be a species of *Tricellaria* new to Britain, and indeed to the Atlantic. It was apparent from the literature that there was some confusion regarding the characteristics of three similar species. The resolution of this involved an extensive literature search and the examination of historical and recent material.

(This Chapter, in somewhat different form, constituted the taxonomic element of 'The distribution, origins and taxonomy of *Tricellaria inopinata* d'Hondt and

Occhipinti Ambrogi, 1985, an invasive bryozoan new to the Atlantic'; Dyrynda et al., Journal of Natural History, 2000, 34, pp.1993-2006).

Chapter 4 is the 'Materials and Methods' section in respect of the central part of the study, which investigated the spatial arrangement of autozooids and heterozooids within a colony of *S. reptans*. This sets out the objectives of the study, and various considerations which were taken into account in efforts to achieve them. It describes in detail a coding scheme which was used to record details of all relevant parameters, and was central to the investigation. The advantages and disadvantages of various approaches to using this are discussed. There is a brief review of the preliminary, detailed and supplementary studies which were carried out.

Chapter 5 introduces, and details the results of, preliminary, detailed and supplementary studies in respect of *S. reptans*. In large measure the results, generally in table form, relate zooids to various parameters of their pattern of occurrence within the colony. Some important results were only apparent as a result of diagrammatic representations. Because the supplementary studies often replicated earlier studies, and because there were certain variations in overall level of occurrence, the results were collated and summarised.

Chapter 6, without a preliminary investigation, details the results of similar studies on *Tricellaria inopinata*. These investigations were carried out to ascertain whether or not characteristics newly described for *S. reptans*, also obtained in a species from a closely related genus, and therefore had a wider applicability.

Chapter 7, the conclusion, discusses the results in respect of both species, in respect of the arrangement of both autozooids and polymorphic heterozooids. The similarities and differences between the two species are highlighted. A very similar colonial structure was identified in both species. Some results, quite clear in themselves, raised further questions as to how and perhaps why they occurred. Other results, perhaps inevitably in an investigation of this nature, although very definite, did not suggest to me any possible explanation for their occurrence.

1.2 INTRODUCTION

The central focus of this thesis is on the structural, and perhaps functional, organisation of two species of arborescent cellularine Bryozoa from closely related genera, *Scrupocellaria reptans* and *Tricellaria inopinata* (Family Candidae) in as much as this is manifested in the spatial arrangement of the constituent zooids within a colony. All bryozoans are colonial organisms, the colony being composed of a number, often a very great number, of individual zooids which – formed by asexual budding – are assumed to be genetically identical. Individual zooids are rarely of only one form, monomorphic, more often occurring in a variety of discrete forms, polymorphic. Overall colony structure and form result from the characteristics of the budding process of the individual species, astogeny, and, to a greater or lesser extent, environmental factors. Evolution acts at the level of the colony, and its overall form and the nature, number and spatial distribution of all its zooids, both autozooids and polymorphic heterozooids, are seen as the result of the evolutionary process.

Essentially I am looking at the spatial relationships between all of the constituent zooids of a colony. Current bryozoan taxonomy is largely based on the morphology of autozooids, although the types of polymorph present, their numbers, morphology and spatial distribution, are also widely used. Information within the literature regarding the latter, especially those polymorphs whose occurrence within the colony is irregular, is invariably qualitative. The arrangement of autozooids, perhaps via intermediate structures such as internodes (branches) or fronds, and the resulting structure and morphology of the colony, are much less frequently utilised taxonomically.

This introduction will therefore need to include a general introduction to the Bryozoa as a group, their general biology and classification. In particular it will concentrate on those aspects of their biology that relate to their colonial nature. The budding process, astogeny; the spatial arrangement of all zooids, autozooids and heterozooids within a colony; colony form and variations in the level of colonial integration.

In order that the colonial nature of bryozoans can be seen in a wider context, it is desirable to discuss briefly the nature of social and colonial life, and the phenomenon of polymorphism, which is often although not always, an important element of both. As a prelude to that discussion it is necessary to say something about the nature of clonal and aclonal organisms, the characteristics of which are of considerable importance in relation to social and colonial life.

1.3 CLONAL AND ACLONAL ANIMALS

The essential difference between clonal and aclonal animals "is that parents and progeny in the former are genetically identical whereas in the latter they are not" (Hughes, 1989). A clone, therefore, "is an assemblage of individuals that are genetically identical by descent" (Bell, 1982). The individual clone mates may exist as separate individuals (unitary) or remain connected in a colony (colonial).

Asexual reproduction may occur in a variety of ways which, in the first instance, may be distinguished by whether they are gametic or agametic. Within the former, i.e. parthenogenesis, the segregated germ line is involved, each offspring develops from a single cell, and female gametes give rise to new individuals without fertilisation by a male gamete. Whether cloning occurs depends on the details of the process. Parthenogenetic eggs may be haploid and develop into males, arrhenotoky, as in hymenopterans; or diploid and develop into females, thelytoky. Diploid eggs may result in cloning but the preservation of the maternal genome depends on how they became diploid (Hughes, 1989). In apomixis meiosis is suppressed, there is a single mitotic division, there is no genetic mixing, and genotypes are identical by descent. In automixis meiosis does occur, and the diploid genome is restored by a variety of mechanisms which may occur before, during or after meiosis. In the first of these, premeiotic restitution, in which chromosome numbers double without concurrent nuclear division (and, assuming that only sister chromatids pair) the process is equivalent to apomixis, and there is no genetic mixing (Bell, 1982). Intrameiotic and postmeiotic restitution both involve genetic mixing (Hughes, 1989). Parthenogenesis of one type or another (excluding rare cases) is known to occur in seven phyla (Hughes, 1989):-

- Gastrotricha
- Rotifera
- Nematoda
- Mollusca
- Arthropoda
- Tardigrada
- Chordata

In agametic asexual reproduction there is no involvement of a segregated germ line, offspring develop from a group of cells, and the production of new individuals occurs in a variety of ways:-

- Polyembryony, the development of a number of embryos from a single zygote, occurs rarely, but is characteristic of cyclostome bryozoans (Harmer, 1893; Ryland, 1996; Craig et al., 1997), and certain parasitic hymenopterans (Askew, 1971).
- Fragmentation due to external forces results, in some animals, in new individuals developing from the fragments, e.g. many echinoderms, some annelids.
- An individual may as a result of undergoing fission; give rise to two. It occurs in many echinoderms (Emson and Wilkie, 1980) without morphological modification; architomy (Hughes, 1989). In other taxa, notably turbellarians and annelids, there is morphological modification prior to fission; paratomy (Hughes, 1989).
- An individual may, by laceration, perhaps repeated, shed small volumes of tissue, each of which develops into a new individual. This occurs notably in acontiate anemones, and in some benthic ctenophores and turbellarians (Hughes, 1989).
- New individuals may arise as a result of the process of budding, the formation of new individuals from primordial tissue by growth and cellular differentiation, without division of the parent (Hughes, 1989). In many instances the newly budded individuals remain physically, and often physiologically, connected to their progenitor, and a modular colony, notably hydroids, bryozoans, composed of a number of zooids, results. [Such

colonies pose questions as to what is an individual (Wilson, 1975) since individual zooids are frequently, although not universally, able to feed and reproduce. From an evolutionary point of view, the colony is the individual on which natural selection acts (but see Tuomi and Vuorisalo, 1989)].

Agametic cloning is known to occur in 13 phyla (Hughes, 1989):-

- Porifera
- Mesozoa
- Ctenophora
- Coelenterata
- Platyhelminthes
- Nemertea
- Annelida
- Sipuncula
- Phoronida
- Bryozoa
- Entoprocta
- Echinodermata
- Hemichordata

There are a number of advantageous consequences resulting from cloning which were summarised by Hughes (1989):-

- High levels of heterozygosity as a result of an absence of recombination and segregation.
- For the same reason well-adapted genomes are perpetuated.
- The reproductive rate may be twice that of taxa which produce males.
- Senescence may be delayed or circumvented as somatic copies are developed from undifferentiated somatic cells.
- For all, but especially for non-colonial clones, the risk of mortality is reduced by the physical separation of the ramets of the genet. A high reproductive rate also facilitates the replacement of losses.
- Cloning avoids the allometry between metabolic rate and body mass.

- Cloning also avoids the declining surface area to volume ratio that otherwise obtains.
- Within a ramet, the fact that modules are isogeneic greatly facilitates the production of non-reproductive polymorphic individuals.

Cloning and sexual reproduction are of course not mutually exclusive and the widespread occurrence of periods of cloning interspersed with episodes of mixis suggests that in evolutionary terms the two are frequently complementary (Lewis, 1987). The absence of cloning in some animals may be due to developmental constraints prohibiting particular cloning pathways. Agametic reproduction requires a body plan that can be subdivided, i.e. Arthropods are prevented from any form of division by their exoskeleton, and vertebrates by their morphological complexity. Alternatively aclonality may result not from any constraint but simply from selection pressures (Hughes, 1989).

Within clonal animals it is taxa in which clone mates remain connected, modular colonies, which will be considered here. Such colonies with mutually interdependent, often polymorphic individuals have an aclonal counterpart in the eusocial insects. A brief consideration of their similarities and differences is I believe, relevant.

1.4 SOCIAL AND COLONIAL ANIMALS

1.4.1 Introduction

'Social' and 'colonial' are terms which, even within the discipline of biology, are used imprecisely and often interchangeably. 'Social' is often used to refer to less than essential associations, and 'colony' is frequently used to describe a group of a species living within the same area, via more structured groups through to groups whose members are obligately mutually interdependent. It is this last characteristic which distinguishes the truly social or colonial animals considered here. Colonies may consist of separate motile individuals, and essentially these are restricted to the social insects. Alternatively they may consist of physically, and probably physiologically connected, non-motile individuals, zooids, which form a collective

colonial structure. The latter are a more heterogeneous group than the social insects and are generally referred to as colonial marine invertebrates.

1.4.2 The eusocial insects

Entomologists concerned with social insects, define the truly social, eusocial, insects, in terms of three basic parameters, to differentiate them from species whose associations are less fundamental (Wilson, 1975):-

- Individuals of the same species cooperate in caring for the young.
- There is reproductive division of labour, with only a limited number of individuals capable of sexual reproduction.
- There is an overlap of at least two generations, and offspring assist their parents for some period of their life.

All eusocial insects are aclonal animals and motile.

1.4.3 The colonial marine invertebrates

The essential characteristics of colonial invertebrates have also been delineated, and the three criteria which need to be satisfied for an organism to be designated 'colonial', put forward by Boardman and Cheetham (1973) are generally accepted:-

- The organism consists of asexually produced modules.
- Modules are physiologically connected, facilitating the sharing of resources.
- There is some degree of colonial coordination of the modules.

As Harvell (1994) has pointed out, 'by this definition the social insects are not strictly speaking 'colonial', since modules are not isogeneic and there are no organic connections among the modules'. Resources are however shared, modules are behaviourally integrated, and the phenomenon of polymorphism, a prominent if not essential characteristic of colonial invertebrates, is also often present. Rosen (1979) regarded insect colonies as "discontinuous modular societies".

To avoid possible ambiguities, colonies of physically connected 'units' are more specifically referred to as modular organisms. For modular colonies there is an aspect to their organization that is absent from colonies of physically separate animals; that is the physical arrangement of the constituent modules or zooids within the colony. This will be considered below in Section 1.6.8.

1.4.4 The taxonomic distribution of eusocial and colonial animals

1.4.4.1 THE EUSOCIAL INSECTS

Species of eusocial insects are only found in two of the 25 orders of insects currently recognised (Margulis and Schwartz, 1987) the Isoptera, the termites, and the Hymenoptera, which includes the bees, wasps and ants.

Before attempting to give some idea of the incidence of eusociality within these groups it must be said that many species remain undescribed. For the ants, the group about which most is known, Holldobler and Wilson (1990) estimated that while ~9,500 species have been described, given a binomial name, probably that number again remain to be described. Further, and perhaps more importantly, the number of species whose behaviour has been investigated to a degree necessary to pronounce on their social organization, is minute. Nevertheless it is generally believed that within the Isoptera, all ~2,000 species are eusocial. In the Hymenoptera the occurrence of eusociality is very episodic. In the smaller of the two sub-orders, the Symphyta, there are no eusocial species. The second sub-order, the Apocrita, is itself divided into two Series, and eusociality is confined to the by far the smaller of the two, the Aculeata which includes the ants, bees and wasps. Eusociality occurs often but episodically. All species of ant (~9,500spp), but only a small minority, ~1,800, of the ~40,000 species of bee, and ~12,000 species of wasp, are eusocial.

1.4.4.2 THE COLONIAL MARINE INVERTEBRATES

Within the 33 phyla in the Kingdom Animalia distinguished by Margulis and Schwartz (1987) coloniality is found in only six:-

- Cnidaria
- Rotifera
- Entoprocta
- Bryozoa
- Hemichordata
- Chordata

The phyla vary considerably in size and in the proportion of species within them that are colonial. Colonial invertebrates are very largely marine and constitute a much more heterogeneous group than the eusocial insects.

Within the Cnidaria both polypoid and medusoid forms exist and some species have both within their life cycle. Coloniality is generally more characteristic of polypoid forms although medusoid forms may also occur within generally polypoid colonies. The vast majority of the Hydrozoa, in which the polypoid form is generally prominent, are colonial. Within the pelagic Siphonophores the colony is composed of both medusoid and polypoid forms (Kirkpatrick and Pugh, 1984). Virtually all Scyphozoa, in which the medusoid form is pre-eminent are solitary, although a minority of the Coronatae (e.g. *Stephanoscyphus*) have a colonial polypoid stage (Werner, 1979; Barnes, 1980). The majority of the polypoid Anthozoa are colonial; all of the octocorals, a variety of different forms, are colonial; and in the Zoantharia, most zoanthids, the majority of scleractinian corals and the antipatharians are all colonial (Barnes, 1980).

Within the Rotifera among the pelagic species there are a small number of colonial forms (Barnes, 1980). The two smaller of the three families within the Entoprocta are colonial (Nielsen, 1989). Pterobranchs, a very small group within the phylum Hemichordata generally live in aggregations, but in *Rhabdopleura* individuals are connected by a stolon (Barnes, 1980). Within the Chordates, colonial forms exist in the Ascidacea and the Thaliacea. In the former, the sea-squirts, there are many colonial species exhibiting a range of levels of integration (Berrill, 1950b; Millar, 1970; Sabbadin, 1979). In the latter all pyrosomids and doliolids are colonial (Fraser, 1981).

The Bryozoa is the only completely colonial phylum, although there is variation in the level of their colonial organisation. This is discussed below in Section 1.6.7.

1.4.5 Why is the taxonomic occurrence of eusociality and coloniality so episodic?

The taxonomic distribution of eusociality and coloniality exhibits an episodic but noticeably clumped distribution. This strongly suggests that while its occurrence is, to some degree, related to certain biological characteristics which are associated with particular taxonomic groups, other elements are also important.

Does the taxonomic distribution of agametic cloning and the forms of parthenogenesis which result in cloning, relate to the taxonomic occurrence of eusociality and coloniality? Whilst agametic cloning is a prerequisite of colony formation, it is clear from the taxonomic distribution of the latter (see Sections 1.3 and 1.4.4.2) that coloniality is of more limited occurrence. The relationship between parthenogenesis and eusociality is more tenuous. Parthenogenesis does not occur in the Isoptera; and in the Hymenoptera, in the form of arrhenotoky, results in haploid males, which are not clonal. The haplodiploid mode of sex determination however may play a role in the occurrence of eusociality (see Section 1.4.5.1.1 below).

1.4.5.1 THE EVOLUTION OF EUSOCIALITY

The occurrence of eusociality is taxonomically clumped on a large and small scale. How it has evolved in this way is of enormous interest, and theoretical explanations and investigative studies have created a vast literature. Nevertheless, no generally accepted conclusions have been reached, beyond perhaps, that no single trait can be invoked as the causative factor. The situation is quite different in the two orders in which it occurs, and a brief survey of the hypotheses advanced, and some of the problems associated with them, must suffice.

1.4.5.1.1 Evolution of eusociality in the Hymenoptera

Because eusociality has arisen at least 11 times in the Hymenoptera, and perhaps only once, in the Isoptera, within all other insects, an explanation was sought within the characteristics of the group. In almost all of the species of Hymenoptera which have been investigated, fertilised eggs produce females and unfertilised eggs males; Dzierzon's rule (Kerr, 1962; Wilson, 1971). This has, via modern genetics, been developed into the concept of the haplodiploidy mode of sex determination; a characteristic rare in the insects.

Hamilton (1964) put forward a genetic theory, which ascribed a central role for haplodiploidy in the evolution of eusociality generally, and in particular the evolution of a non-reproductive worker caste. In essence his theory said that for an altruistic trait to evolve, the sacrifice of fitness by an individual must be compensated for by an increase in the fitness in certain relatives, by a factor greater than the reciprocal coefficient of relationship to these relatives. The concept of inclusive fitness. The coefficient of relationship is the equivalent of the average fraction of genes shared by common descent. The haplodiploid mode of sex determination results in the coefficient of relationship among sisters being 3:4, while between mother and daughter, it is 1:2. It follows from this that female offspring may increase their inclusive fitness more by caring for their younger sisters than by rearing her own offspring. According to the theory, hymenopterans should, other things being equal, tend to become social (Wilson, 1971).

A number of entomologists have warned against overemphasis on the 3:4 relatedness hypothesis (Alexander, 1974; Lin and Michener, 1972). Hamilton himself, and Wilson, both recognised that the theory cannot, in itself, completely explain eusociality in the Hymenoptera. According to Andersson (1984) "at least five lines of evidence cast doubt on the overwhelming importance sometimes ascribed to haplodiploidy". Primarily, whilst all hymenopterans are haplodiploid only ~8% of species are eusocial, and eusociality is characteristic of all termites which are diploid. Hamilton's theory also makes a number of specific assumptions regarding single

male mating, a single queen, and the sex ratio of reproductives actually produced within a colony, which may not actually be met (Andersson, 1984).

Trivers and Hare (1976) in respect of the sex ratio of reproductive offspring believed that if Hamilton's theory was correct, the sterile workers' inclusive fitness would be best served if the ratio of reproductive females to males was 3:1, whilst for queens the optimum ratio would be 1:1. Using data from a number of previous studies, predominantly on ants, they felt the results generally approximated to a 3:1 female to male ratio.

The matter remains contentious, but indirect kin selection, the propagation of genes through relatives rather than offspring, has probably been important in the evolution of eusociality in the Hymenoptera, whatever the importance of haplodiploidy may prove to be (West-Eberhard, 1975; Wilson, 1975; Sudd and Franks, 1987).

1.4.5.1.2 Evolution of eusociality in the Isoptera

All 2,200 known extant species of termite are believed to have sterile castes (Andersson, 1984). Termites are diploid, and workers are of both sexes. Several hypotheses have been put forward to explain their eusociality.

- The symbiont hypothesis. Termites require symbiotic intestinal Protozoa to digest cellulose, and as these are lost with each moult, termites must obtain new Protozoa from other colony members, which they do by anal trophallaxis. This requires social life until adulthood, but does not necessarily lead to eusociality.
- The inbreeding hypothesis. It has been suggested that inbreeding may lead to high degrees of relatedness within colonies and thus facilitate eusociality (Bartz, 1979).
- Chromosomal linkage hypothesis. In several termites part of the genome is propagated as a sex-linked complex. Lacy (1980) suggested that this increased relatedness among brothers and among sisters, reduced brother-sister relatedness, and could lead to termite workers favouring their own sex, which might facilitate the evolution of sterile workers.

1.4.5.1.3 Preconditions favouring eusociality in the insects

The selective mechanisms above, considered on their own, do not appear to explain why eusociality has evolved within the insects, in the way it has. The evolution of eusociality has no simple causality. What preconditions have facilitated eusociality?

A number of traits, favouring eusociality have been identified;-

- Nest building and repeated food provisioning (Hamilton, 1972).
- Defence of offspring (Evans, 1977).
- Overlapping adult generations (Wilson, 1971).
- Mutualism (Lin and Michener, 1972; West-Eberhard, 1975).
- Manipulation of daughters by queens.

All of the above may be steps on the way to eusociality but they do not explain the evolution of a sterile worker caste.

1.4.5.2 THE EVOLUTION OF COLONIALITY

All colonial invertebrates as defined above are clonal animals, and the evolution of coloniality is very different from that of aclonal unitary animals evolving to eusociality. It is essentially the non-separation of clone mates and the development of their mutual interdependence. The concepts underlying colony formation and development in colonial marine invertebrates were formulated and reviewed by Beklemishev (1969) in his 'Comparative anatomy of invertebrates', who concluded that the development of colonies progressed in three major ways.

- By a reduction in the individuality of individual zooids.
- By an increase in the individuality of the colony.
- By the development of cormidia, regular groupings of various zooid forms within the colony.

Beklemishev distinguished a number of aspects to each of these and in many cases described a series of intermediate stages in the development of true colonial organisation.

Firstly, the level of an individual zooid's individuality, their degree of independence vis a vis the colony, which involves a number of different parameters. The nature and extent of the organic connections between zooids is clearly very important. The permanence of the links between individual zooids is fundamental and in true colonial marine invertebrates, either all individuals remain connected or, in certain groups, only certain specific forms become detached. The size and structure of zooids within a colony is reduced relative to individuals of related free-living species. Within colonies, the lifespan of the colony is generally greater than that of many of its constituent zooids, which may undergo complete or partial degeneration or regeneration, e.g. the polypides of many bryozoans. Polymorphism, the development of different zooid morphologies within a single colony, related to division of labour within it, is an important characteristic of many, but by no means all colonial forms, as will be discussed below. The extent of its occurrence varies considerably and in extreme cases, such as the various kenozooids of the Bryozoa, individual zooids may be unable to feed or to reproduce. In some colonial groups the morphology of a zooid may be determined by its precise location within the colony. Finally, the partial or total dissolution of zooids may occur as a result of the requirements of the colony.

Secondly, there is the increase in the individuality of the colony, which may occur in a variety of ways. The formation and development of a coenosarc is a major aspect of the colonial nature of some colonial groups. The creation of colonial organs, sometimes necessary for a colony – which is much larger than an individual zooid – may occur in various ways. It may be achieved by the specialisation and enhancement of the functions of certain zooids, polymorphism, or by the creation of organs through the merging of a number of zooids. The development of physiological integration in a colony is of fundamental importance in colonial invertebrates, and this involves some form of colonial circulatory apparatus. Increasing colonial control over its constituent zooids is observable in variations in a number of characteristics of colony growth and form. This may involve a particular growth sequence, such as the branching pattern of certain arborescent hydrozoans and bryozoans, or a strictly determinate growth sequence. It may manifest itself in the final shape and/or form of the colony, and in some largely motile colonies, it may be a colony imposed

symmetry of form. Related to this are degrees of complexity in the growth of the colony, largely manifested in differences in the morphology of zooids in different generations of the colony, but which may also involve changes in the pattern of growth as the colony develops. The extent of the colonial phase, within a life cycle which involves sexual and asexual reproduction, and hence includes a solitary phase, is also indicative of the level of colonial control. Asexual reproduction may occur earlier or later, and colonial life occupy a greater or lesser proportion of the whole. The capacity of certain colonies to produce new colonies, by division or budding or the production of stolons, is indicative of the primacy of the colony.

Thirdly, there is the development of cormidia, 'colonies within colonies', groups of zooids of different morphologies, which occur regularly, and which jointly fulfil several different functions. Beklemishev felt that the formation of cormidia played a minor role in the development of coloniality, compared to the other two processes.

The above theoretical approach was centred on levels (zooids, cormidia and the colony) and degrees of individuality, in relation to the physical structure of a colony. Mackie (1986) offered a different perspective stressing physiological and behavioural aspects of coloniality. Temporary colonies could initially form in clonal animals whenever the rate of bud production exceeded that of bud separation. Such colonies may well have possessed a competitive advantage, in terms of substrate colonisation, over separate individuals, clonal or unitary. The existence of primary tissue connections could facilitate the transfer of metabolites, and this in turn the possibility of non-feeding zooids fulfilling specialised functions. Further it could facilitate the transmission of behavioural responses to external stimuli throughout the colony and thus a colony-wide response.

Mackie (1986) also noted that in relation to modular colonies, that it was the characteristics of sessile colonies (the vast majority of colonies are sessile) that were generally considered. Such colonies often exhibit indeterminate growth and the ability to grow rapidly. As a result some achieve a very large size, and for those species which are able to regenerate from fragments, great longevity. Modular growth frequently results in a branching structure that automatically 'regulates

competition between zooids' (Knight-Jones and Moyse, 1961) and is presumably the most effective arrangement for feeding (Bayer, 1973). Plasticity of size and form is characteristic. Pelagic colonies are generally very different; they do not exhibit indeterminate growth, branching is uncommon and colonies show little plasticity. As a result of their motility however, some are amongst the most highly integrated of forms (Mackie, 1986).

Variations in the level of colonial integration in the Bryozoa are discussed below in Section 1.6.7.

1.5 POLYMORPHISM

1.5.1 Introduction

Polymorphism of complete organisms within the kingdom Animalia is, if we exclude sexual dimorphism, of infrequent occurrence. It is essentially concentrated in the aclonal terrestrial social insects, and the clonal, aquatic, largely marine, colonial invertebrates. In both groups their social or colonial character involves the obligate mutual interdependence of the individual members of the colony. This may involve division of labour, which may be achieved, especially in motile aclonal animals by polyethism, or may involve, especially in sessile clonal animals, polymorphism. There are great differences between the two groups, most notably in how the polymorphic individuals are produced, and indeed how all 'individuals' are produced! (As discussed in Section 1.3).

Few accounts consider polymorphism as a phenomenon; that is in both the eusocial insects and the colonial invertebrates, and Harvell's (1994) comprehensive review of the topic is very useful. The generally episodic occurrence of eusociality within the insects, and coloniality within the kingdom Animalia, is also apparent in respect of polymorphism, which exhibits an even more restricted distribution.

1.5.2 Taxonomic occurrence of polymorphism

1.5.2.1 THE EUSOCIAL INSECTS

Polymorphism has evolved only sporadically in the eusocial insects. Within the Isoptera generally, polymorphism is much more prevalent within the soldier than the worker caste. Within the higher termites however polymorphism within the worker caste is more developed (Wilson, 1971). Within the ants, where polymorphism exhibits the greatest structural differentiation between castes, the differentiation of workers into sub-castes, minor and major workers (soldiers) is uncommon. Of 263 extant genera, in only 44 are there species with a prominent degree of polymorphism within the worker caste (Oster and Wilson, 1978). Worker sub-castes are essentially absent in the bees and wasps.

1.5.2.2 THE COLONIAL MARINE INVERTEBRATES

In only three of the six phyla which contain colonial species, the Cnidaria, Bryozoa, and the Chordata (within the Tunicata) is polymorphism a notable feature. Its occurrence is episodic in both the Cnidaria and the Chordata, and in the Bryozoa where its occurrence is most frequent, it is not universal.

Within the Cnidaria, polymorphism is absent from the Scyphozoa, rare in the Anthozoa, and well developed in the orders Hydroida (benthic) and Siphonophora (pelagic) within the Hydrozoa. It is essentially absent from the other orders of the class.

Polymorphism is present in only one of the three classes within the Tunicata, the thaliaceans. Salps exist in solitary and aggregate forms, but true polymorphism is only present in the doliolids.

Polymorphism within the Bryozoa will be discussed in detail within the section on bryozoans below. The level of occurrence varies greatly in extent within the different classes, and indeed at all taxonomic levels.

1.5.3 Why is the taxonomic occurrence of polymorphism so episodic?

The episodic nature of polymorphism, within the groups in which it occurs, poses the obvious question as to the factors underlying this. In respect of the colonial marine invertebrates several explanations have been put forward.

1.5.3.1 THE OCCURRENCE OF POLYMORPHISM IS RELATED TO EVOLUTIONARY STATUS

Firstly, there is the argument that since polymorphism is a derived character (Cheetham and Cook, 1983) found largely in the most derived clades of the various colonial groups in which it occurs, that this in itself could account for the observed pattern of polymorph occurrence. There is much evidence in support of this, but there is some danger of a circular argument, when presence or absence of polymorphism is used in drawing up phylogenies (Harvell, 1994). Within the Cnidaria, the paucity of polymorphism in the scleractinian corals could be explained thus, and the primitive position of the Anthozoa, in which polymorphism is rare, within the phylum, would appear substantiated by its possession of linear rather than circular mitochondrial DNA (Bridge et al., 1992). However polymorphism is characteristic of the Pennatulacea within the class. Within the hydroids polymorphism is rare in the Athecata and much more developed in the Thecata (Petersen, 1979). Within the Bryozoa, as discussed below, polymorphism is completely absent from the Phylactolaemata, and it is also not well developed in the Cyclostomatida and the Ctenostomatida, both of which are considered more primitive than the Cheilostomatida, in which polymorphism is much more fully developed. Stratigraphical evidence supports the view of an earlier origination of the ctenostomes than the cheilostomes (Cheetham and Cook, 1983). Within the chordate Tunicata, polymorphism is completely absent from the ascidians, which Berrill (1950b) considered the most primitive class of the group, and is present only in the thaliaceans. In general the absence of polymorphism in certain colonial marine invertebrates could well be a plesiomorphic condition. Cladograms based on molecular characters are necessary to substantiate this (Harvell, 1994).

1.5.3.2 COMPARTMENTALISATION

It is arguable that for specialisation of individuals to develop it is necessary to have separate compartmentalised individuals. (Ryland (1979) has pointed out that the advantages of compartmentalisation would be nullified if there was no system for the efficient transfer of metabolites). Within the Bryozoa polymorphism is only completely absent from one admittedly very small class, the Phylactolaemata. The autozooids of this class are unlike those of all other bryozoans in that they share a continuous body coelom, and in that respect are not separate zooids (Ryland, 1979). There is some disagreement concerning when zooids are, and are not, separate. According to Harvell (1994) species of thecate hydroids and siphonophores are highly integrated and polymorphism is very highly developed, although they are the least compartmentalised of any colony, their "polyps sharing a common gut". Mackie (1986) states that "in cnidarian colonies the gut cavity is continuous throughout the colony and transport of nutrients is simple and rapid". (Rees (1971) found such rapid transport in the hydroid *Pennaria*, and Mackie and Boag (1963) found it in a siphonophore). A very different view of the degree of separation of zooids in thecate hydroids is expressed by Cornelius (1995). He refers to the basal sphincter at the junction of the hydranth and the coenosarc, and to the most obvious function of the coenosarc as facilitating the transfer of metabolites between zooids. He states "the coenosarc is 'common property', belonging to no one polyp in the colony but to them all". From this perspective the coenosarc is not a 'common gut' but a rapid transport system analogous to the bryozoan funiculus.

1.5.3.3 ENERGETIC COSTS

It has been argued that energetic costs, since non-feeding polymorphs are by definition a nutritional drain on the colony, could be a constraining factor. Harvell (1994) discussing this, said that if this was the case she would have expected species which enjoyed an energy subsidy from endosymbiotic photosynthetic algae, such as scleractinian corals, to be at an advantage relative to organisms such as hydroids and bryozoans which did not. I am not sure this is a valid argument since it would surely be the total available energy rather than its source that would be the crucial factor.

There is clearly an energetic cost to the production of heterozooids but costs and benefits are not easily, and have not been, quantified (Harvell, 1994). Colonies of *Membranipora membranacea* in the field in which spines were induced by weekly exposure to nudibranch extract grew more slowly than control colonies (Harvell, 1992), and the costs of spine production also include reduced reproductive rate (Yoshioka, 1982).

1.5.4 Factors facilitating the occurrence and maintenance of polymorphism in colonial marine invertebrates

Harvell (1994) suggested that the high incidence of polymorphism in some colonial marine invertebrates results from, firstly, unusually high origination rates and, secondly, strong natural selection favouring division of labour in colonies constituted of isogeneic modules.

1.5.4.1 HIGH ORIGINATION RATES

Harvell argued that high origination rates of morphological novelties are facilitated by four features:-

- the iterated developmental process.
- the late differentiation of the germ line.
- the lability of signal transduction pathways.
- the potential for partially functioning "hopeful monsters" to be nurtured by the colony.

Harvell also felt that origination rates of morphological novelties in colonial marine invertebrates may be high due to their propensity for environmentally induced heterochronic shifts, and that genetic assimilation of environmentally induced variants could be facilitated by the late differentiation of germ cells and their redifferentiation in each newly budded zooid.

1.5.4.2 PHENOTYPIC PLASTICITY

Phenotypic plasticity may facilitate and promote the origination of novel forms (West-Eberhard, 1989) and the propensity for plasticity in the colonial invertebrates

may dispose them to high origination rates of such novelties. High phenotypic plasticity is associated with inbreeding, producing low heterozygosity (Lerner, 1954; Palmer, 1986) and many colonial marine invertebrates are likely to be inbred, due to very limited larval dispersal. Philopatry and inbreeding may prove to be characteristic of colonial benthic invertebrates (Jackson, 1986). Hughes (1989) suggested that these traits may not be side-effects resulting from short-distance dispersal, but result from selection pressures, given the variety of mechanisms restricting larval dispersal in different taxa. Harvell (1994) suggested a possible link between families with planktotrophic larvae and hence long distance dispersal, and low levels of polymorphism. It is of course also true that planktotrophic larvae are considered the plesiomorphic condition.

1.5.4.3 STABLE ENVIRONMENTS

The idea that polymorphism would be facilitated by stable environments and constant conditions seems reasonable and has been widely accepted (Schopf, 1973; Moyano, 1982). However as Hughes and Jackson (1990) observed, the evidence is not completely convincing in several respects. The idea derived in large part from Wilson's (1968) 'The ergonomics of caste in the social insects'.

Wilson addressed the problem of why the ratios of the castes in a colony of social insects varied between species. With the assumption that natural selection occurred at the level of the colony he felt that, "this matter of the presence or absence of a given caste, together with its relative abundance when present, should be susceptible to some form of optimisation theory". A preliminary Linear Programming Model resulted in a number of predictions. Amongst these were firstly, that "as long as contingencies occur with relative constant frequencies, it is of advantage for the species to evolve so that in each mature colony there is one caste specialised to respond to each kind of contingency". Secondly the model predicted that, "in a constant environment, caste determination should evolve so that each caste became increasingly specialised to its single assigned task". He recognised that observed levels of polymorphism within the social insects were lower than would be expected on this basis. This led to his proposing that there must be opposing selection

pressures, the most obvious of which were fluctuating environmental conditions. A survey of the level of polymorphism occurrence in a number of regional ant faunas broadly demonstrated that the level of polymorphism decreased the further one travelled from the tropics. There were exceptions but he concluded that the results were consistent with the prediction from the ergonomic theory.

Schopf (1973), on the basis of Wilson's model and his study of a number of taxonomic papers, investigated polymorphism within the Bryozoa. He reviewed a number of regional bryozoan surveys within the literature, in respect of the species present, and the extent of their polymorphism, in three very different environments:-

- The tropical West Atlantic, <125 metres deep.
- The American Arctic, <150 metres deep.
- The Atlantic deep sea, >2000 metres deep.

He also investigated a number of estuarine species.

Schopf considered that the first and third of the above were more stable, constant environments, than the second and the estuarine habitats.

The stability or otherwise of environmental conditions, in as much as they affect bryozoans, is not simply a matter of latitude, as Schopf recognised. Unfortunately he did not consider all polymorphs, confining his attentions to avicularia and vibracula, and the number of these forms a species possessed. The results, in terms of the percentage of species with one polymorph, within the different environments, were not completely as anticipated. Although none of the 10 estuarine species had even one polymorph (not unexpected) the percentage occurrence within the three other environments was remarkably similar, at 75%. When the occurrence of two or more polymorphs was considered, the incidence was higher in the tropical West Atlantic than in the other two environments, by 50%. However, the rate of occurrence in the shallow Arctic was very similar to that in the deep Atlantic. The results were therefore, in my opinion, inconclusive. This may be due to variations in sample size, an inaccurate assessment of the stability or otherwise of environmental conditions (in as much as they affect bryozoans) or perhaps, because the overall incidence of

polymorphism was not considered, and perhaps indeed, cannot easily be quantified. Nevertheless Schopf felt that they confirmed the predictions of the theory.

Other studies, including (Moyano, 1982), have also concluded that environmental stability promoted zooidal polymorphism. This view was strongly challenged by Hughes and Jackson (1990). They attempted to quantify a more limited range of abiotic environmental parameters rather than classifying environments in broad terms. They also adopted a more systematic quantified approach to morphological variation which included all polymorphs not just avicularia and vibracula. They analysed the distribution of polymorphism in cheilostomes in a number of regional studies and concluded that, "it provides no evidence for a causal relationship between habitat constancy and morphological specialisation at the zooidal level". They went further, that "the analogy between zooidal polymorphism and the caste system in social insects (Schopf, 1973) may not be as close as was originally believed".

Cheetham (1973) in his study of polymorphism in *Poricellaria* and *Vincularia*, observed the opposing trends (one increasing and one decreasing) which the two species exhibited in this respect. Much of the material studied was from sympatric species of the two genera, and he observed that if Schopf's claim was valid, the two genera must have been responding to different facets of the same environment.

1.5.5 Mechanisms by which polymorphs develop and are maintained

1.5.5.1 HETEROCHRONY

Heterochrony was defined as a change in the timing or rate of a developmental event relative to that of an ancestor (Gould, 1977). Alberch et al. (1979) defined separate parameters for growth rate changes, and variations in the timing of growth initiation and offset. They distinguished descendents, relative to their ancestors, with reduced morphologies, paedomorphic, from those with enhanced morphologies, peramorphic. The former can result from a decrease in growth rate (neoteny), early growth offset (progenesis) or delayed growth onset (post-displacement). Peramorphic morphologies can result from increases in growth rate (acceleration), early growth

onset (hypermorphosis) or delayed growth offset (pre-displacement). Actual morphological changes may involve more than one of these and their individual contributions may be very difficult to identify (Harvell, 1994).

Heterochrony is a very important mechanism by which morphological variations – polymorphs – arise, although environmental cues may be a necessary prerequisite for their actual occurrence.

1.5.5.2 GENETIC ASSIMILATION

Waddington (1953, 1959), working on *Drosophila*, described how initially inducible variants could become genetically fixed through a process he termed 'genetic assimilation'. By selecting for the propensity for the production of a trait in specific environmental conditions, he demonstrated that eventually individuals could be produced that developed the trait across a wide range of environments. An inducible characteristic became constitutive.

1.5.6 Polymorphism in the social insects

Within the eusocial insects polymorphism has been defined "as the coexistence of two or more functionally different castes within the same sex...and they must be stable during one or more instars" (Wilson, 1971). Within the eusocial Hymenoptera they are stable throughout the adult instar.

1.5.6.1 THE HYMENOPTERA

There is great variation in the extent to which polymorphism occurs and in only a minority of ant species is it very fully developed. The situation is different in the different groups, most notably between the ants on the one hand and the bees and wasps on the other. No bees or wasps have developed a well-defined worker sub-caste.

Within the ants three basic castes are found, worker, soldier and queen. Soldiers are often referred to as major workers, and where this is the case, the smaller coexisting workers are described as minor workers. In only a minority of species, are all three female castes to be found together. The worker caste has been lost in socially parasitic species and queens replaced by workers or worker-like forms. Intermediate castes may develop in certain species, i.e. ergatoynes, between queen and worker, and media workers, between major and minor worker. Evolution has led to derived forms of certain castes, which bear little resemblance to the ancestral type, e.g. the very large queens of the army ants (Wilson, 1971).

Although much division of labour in the ants is achieved by temporal polyethism some is related directly to caste. Males, beyond inseminating reproductive females, contribute virtually nothing to the labour of a colony. The behaviour of queens, beyond their production of the brood of the colony, varies with the nature of the society of the species, with those of the more primitive species fulfilling, at least initially, a greater range of roles. As colonies develop the range of activities generally decreases, and in the physogastric condition the queen's activities become limited to locomotion, egg laying and feeding (Wilson, 1971).

The morphological modifications of the soldier caste are generally so extensive that these individuals function almost exclusively in the defence of the colony. The modifications to the head and/or mandibles adapt them to one of three defensive techniques. Firstly, and most commonly, the mandibles may be typical but large and powerful, and the head as a result of the necessary musculature, massive. Such soldiers are adapted to penetrating integument and severing limbs, shearing. Secondly mandibles may be pointed and sickle-shaped or hooked, adapted to piercing. Thirdly, the head itself may be shield-shaped, adapted simply to blocking an entrance to the nest (Wilson, 1971).

Temporal polyethism, the division of labour by age, occurs much more frequently than caste polyethism, division of labour correlated with anatomical differences, polymorphism (Hölldobler and Wilson, 1990). There are a number of worker sub-castes, and in the small number of species studied, types and patterns of occurrence

vary considerably. "Although the degree of polyethism is loosely correlated with the degree of polymorphism, the patterns of the two phenomena cannot be said to be linked in any consistent way among the genera" (Wilson, 1971). Caste, and temporal polyethism are not always easily separated. Knowledge concerning the latter relates to only a tiny minority of genera and species and an overview of the subject lies in the future (Hölldobler and Wilson, 1990).

In the bees and wasps, polymorphism is limited to dimorphism between queens and workers, and even this is absent from species forming small colonies, and only occurs (and increasingly so) in species that form very large colonies. Temporal polyethism exhibits a similar pattern of occurrence (Wilson. 1971).

1.5.6.2 THE ISOPTERA

Within the termites generally, the caste system is remarkably similar, given the taxonomic distance between the two groups, to that of the ants. The worker caste is very similar morphologically from species to species, but behaviourally versatile. The soldier caste is morphologically, through head and mandibles, and behaviourally, specialised for the defence of the colony. In the higher termites however there is a greater incidence of polymorphism within the worker caste (Wilson, 1971).

The caste system of termites differs from that of the Hymenoptera in a number of respects. The neuter castes are constituted of both sexes. In the lower termites there is no true worker caste and worker tasks are performed by nymphs and pseudogates or 'false workers'. In the higher termites work is performed solely by a true worker caste and nymphs and other immature forms are non-functional. The soldier caste is morphologically modified for defence and this may involve large powerful mandibles or 'stopper-like' heads. In more highly developed soldiers physical gives way to chemical defence, and involves hypertrophied glands capable of discharging large quantities of defensive secretions. In some species in the Nasutitermitinae the mandibles have been lost and replaced by a fontanellar 'gun', capable of projecting these secretions some distance (Wilson, 1971).

Studies on temporal polyethism, as compared with caste polymorphism have scarcely begun (Wilson, 1971).

1.5.7 Polymorphism in the colonial marine invertebrates

Within the Cnidaria, the existence of polymorphic forms is to some degree underlain by the simultaneous production of two zooid types, the polyp and the medusa, which, in the plesiomorphic condition, occurred sequentially within the life cycle (Berrill, 1949; 1950a). In the less derived hydrozoans, colonies are dimorphic with a monomorphic sessile polyp stage and a pelagic medusoid stage. However, in forms with well-developed polymorphism there may be several different polymorphs derived from each of these. The most numerous form are the polypoid hydranths which capture and ingest prey. In most species these also function in defence but in some athecate species, special elongate dactylozooids are also present. These are of two kinds, spiral zooids, with numerous nematocysts, which presumably have a defensive function; and tentaculazooids which are thought perhaps to possess a chemosensory capability (Cornelius, 1995). Many thecate species possess nematophores, non-feeding polyps with a defensive function. Whilst in athecates hydranths both feed and bud the reproductive medusae, in thecates these are produced on gonangia. Medusa may be liberated but are more usually retained.

Within the pelagic siphonophores, perhaps the most integrated of all colonial forms, polymorphism is highly developed and involves modified polypoid and medusoid individuals (Mackie, 1986). The types present vary within the group but include polypoid feeding gastrozooids, and modified forms of these, palpons, whose function may be sensory or excretory (Kirkpatrick and Pugh, 1984)! Medusoid forms include nectophores, agents of locomotion, and gonophores, reproductive zooids.

Within the Anthozoa polymorphism is poorly developed. In the Pennatulacea, sea pens, colonies consist of an axial polyp, which buds from its upper end two forms of secondary polyps, tentacled autozooids, and siphonozooids, modified, generally tentacle-less inhalant polyps (Manuel, 1981).

Within the Tunicata polymorphism is a notable characteristic of the doliolids, the various polymorphs fulfilling a variety of roles in a very complicated life cycle (Berrill, 1950b; Hardy, 1959; Fraser, 1981).

Polymorphism is generally of episodic occurrence taxonomically, on a large and small scale. Where it does occur its occurrence is often at a low level but in a small minority of groups its development is very extensive.

1.5.8 Polymorphism in the eusocial insects and the colonial marine invertebrates: a comparison

Clearly eusocial and colonial life, in which specialised morphologies of individuals, often unable to perform certain essential functions for themselves, is a prerequisite for the existence of complex polymorphisms. Only in such obligatory mutually interdependent groups are such individuals viable, and only in such groups, is it feasible for the colony to benefit from the activities of such individuals (Harvell, 1994).

Polymorphism, in the groups in which it does occur, is much more extensively developed in the colonial marine invertebrates than in the eusocial insects. What differences between the two groups may underlie this?

- Firstly, zooids of colonial marine invertebrates are isogeneic and there is therefore no sacrifice of reproductive potential for zooids which do not reproduce. It may well be that for haplodiploid Hymenopterans this may also be the case, at least to some degree. This is less likely for the termites, but see Lacy (1980) who suggested a possible haplodiploid analogy.
- Secondly, division of labour, which underlies polymorphism within an obligately social or colonial organisation. This may be achieved behaviourally, by temporal polyethism, and/or morphologically, by polymorphism. Behaviour is a more pervasive activity in the life of colonies of motile individuals and division of labour within the eusocial insects may well be achieved by temporal polyethism. Within the colonial marine invertebrates, notably the Bryozoa, many polymorphs have no behaviour, they are defined by their morphology.

- Thirdly, within the motile eusocial insects, all individuals, however specialised they may be, have still to be motile, which puts some limits on the degree of their polymorphism. Within the zooids of colonial marine invertebrates there are no such constraints.
- Fourthly, whilst some polymorphic social insects may be unable to feed, and are fed by other colony members, they still need to be able to metabolise their food. Within the colonial marine invertebrates the metabolised products of feeding zooids can be made available to non-feeding heterozooids. The scope for polymorphism is thus increased.
- Fifthly, within many colonial marine invertebrates, notably cheilostome bryozoans, the scope for morphological variation is enhanced by the various structural roles open to them within the architecture of a colony.

1.5.9 Cues inducing the development of polymorphs

1.5.9.1 THE EUSOCIAL INSECTS

Much is known about the development of polymorphism in eusocial insects, particularly in respect of the ants, and the following just touches on a complex topic. Different types of polymorphism are produced by different growth transformation rules. The simplest mechanism involves small differences in initial larval size being transformed into large differences in final size by correlated growth rate changes. A more complex mechanism involves threshold levels, a larva becoming a minor or a major worker as a result of its size at a certain period in its growth (Oster and Wilson, 1978). All transformations are governed by rules which operate during larval growth and adult development within the pupa. Intrinsic control of polymorphism occurs via the levels of two hormones which affect transitions between instars. High levels of ecdysone and juvenile hormone maintain the larval stage, and a drop in the level of the latter is necessary for metamorphosis to the next stage. There is interaction between intrinsic and external cues in the control of polymorphic transformations. In *Myrmica rubra* only larvae which have undergone winter chilling in the last larval stage can become queens. Polymorphic transitions are regulated by six potential types of cues; larval nutrition, winter chilling,

temperature, caste self-inhibition, egg size and the age of the queen (Oster and Wilson, 1978).

1.5.9.2 THE COLONIAL MARINE INVERTEBRATES

The Bryozoa will be considered in Section 1.6.4. Very little is known about cues inducing the development of polymorphs in the Tunicata. This section will deal briefly with such cues in respect of hydroids.

As in the ants considered above, intrinsic and extrinsic factors are involved. Inhibitory and activating morphogenetic factors control the allocation of growth to stolons and hydranths (Műller et al., 1987). Inhibitory substances are produced by hydranths, limiting the proximity of developing hydranths to a minimum distance from existing ones. In *Hydractinia*, the stolon tip produces Proportion-Altering Factor, which spreads proximally in decreasing concentration, and prevents stolon formation in its immediate vicinity (Műller and Plickert, 1982). Also in *Hydractinia* stolon branching is induced by a morphogenetic inducer, Stolon-Inducing Factor (Lange and Műller, 1991).

In respect of extrinsic cues, in athecate hydroids, a change from a stolon, to a hydranth and gonangium dominated colony can be triggered by a change in temperature (Braverman, 1974). In *Hydractinia echinata*, nematocyst-filled stolons proliferate at the margins of contacts with conspecifics, but only at non-self contacts (Ivker, 1972).

1.6 BRYOZOA

1.6.1 Introduction

[A glossary, defining terms, whose use is largely restricted to bryozoans, begins on page 345].

Bryozoans are coelomate, sessile, filter feeding, aquatic (largely marine) colonial invertebrates. Colonies consist of a number, often a very great number, of zooids, all

of which arise by budding from the first individual, the ancestrula. This arises by metamorphosis of a sexually produced larva, which initially is planktonic and which settles on a substratum prior to metamorphosis. Colonies vary enormously in size and morphology, but all are clonal modular organisms; the modules, individual zooids, arise by asexual budding and are assumed to be genetically identical (Ryland, 1979; Thorpe, 1979; Hughes, 1989). The extent and importance of any mutations occurring during modular replication (Buss, 1985; Carvalho, 1994) are not known (Porter et. al., 2000). Intraspecific colony fusion and chimerism are known to occur. Craig (1994) using settlement plates amongst a dense population of *Fenestrulina* sp., found skeletal fusion in 70% of paired colonies and evidence of physiological integration, although the extent to which this was restricted to closely related genotypes was not known. [Interestingly, for ascidians, which have been investigated more extensively in this respect, results are variable. Fusion of colonies of *Botryllus* is restricted to closely related colonies (Oka and Watanabe, 1960; Scofield et al., 1982). However, Bishop and Sommerfeldt (1999) in respect of *Diplosoma listerianum*, found that chimera formation was not dependent on close relatedness. Further Sommerfeldt et al. (2003) concluded that much natural chimerism in this species was attributable to colony fusion].

In the simplest colonies the individual zooids are all identical morphologically, but the vast majority of bryozoan colonies exhibit polymorphism to some degree. It is possible to divide the zooids of a colony into two types on the basis of two different sets of criteria, which are often confused because the resulting division is very similar. Firstly, it is possible to distinguish those zooids, autozooids, which are able to collect food and metabolise it on the one hand, from those which are unable to do so, the heterozooids. These necessarily derive their nutrition, via the funiculus which connects all zooids within the colony, from the feeding autozooids. Secondly, one can distinguish between individuals which have the morphology of autozooids from those which do not, polymorphic zooids. All heterozooids are, by definition, polymorphic, and constitute the vast majority of polymorphic zooids, but some autozooids are also polymorphic, notably in respect of the form of the lophophore of their polypide. Polymorphic zooids are therefore generally unable to feed, as they lack a food collecting apparatus; or digest, since they have no gut. Clearly such

zooids are, nutritionally, a drain on the colony, and it is assumed must be of sufficient utility to the colony to justify their occurrence and maintenance.

The occurrence of heterozooids requires that metabolites can be transferred from feeding zooids. In all bryozoans there is the presence of the funiculus, a network of mesenchymatous tissue strands, although the extent of its development and the functions it performs, varies considerably between the higher taxonomic groups. Within the phylactolaemates and stenolaemates its development and functions are very limited in comparison to the gymnolaemates, in which it is a genuinely colonial network (Ryland, 1979). Bobin (1977) described what she termed the 'funiculi-rosettes complex' as a unique anatomic system, "specialised for exchanges", which resolves "the problem of the extreme compartmentalisation of a single colony community". It is important in transporting metabolic products to non-feeding polymorphic zooids (Bobin, 1977; Hayward and Ryland, 1998).

A generalised description of the morphology of an autozooid provides a necessary benchmark against which the morphologies of the various polymorphs can be compared. Autozooids are generally box, or more rarely flask shaped, measuring <1mm in their longest dimension. The body wall may be uncalcified (in one order) or more usually calcified to some degree. Each zooid consists of a cystid, cellular and skeletal layers of the body wall, and a coelom within which there is a polypide. This is a food gathering apparatus (a crown of ciliated tentacles, the lophophore) its associated musculature, and a gut; all of which generally undergo periodic degeneration and regeneration. Although there are differences between the higher taxa regarding the mechanism of lophophore eversion and retraction, all involve changing the hydrostatic pressure within the zooid. In the cheilostomes, the presence also of an operculum, or hinged flap within the frontal wall, which closes the orifice after lophophore retraction, is of great importance in relation to the polymorphs of that order (Hincks, 1880; Harmer, 1901; Silén, 1977).

Polymorphic zooids arise by heterochrony (see Section 1.5.5.1), a variation of the developmental process which may result in a morphological feature not developing at all, or developing to a lesser or greater extent than is normally the case. Polymorphic

zooids, whatever their morphology, are variations on a theme, the morphology of the characteristic autozooid of that species, which sets constraints on what is possible.

1.6.2 Classification

Bryozoan classification has undergone considerable revision in recent time, but this has generally been below the level of 'order', and the higher taxonomic groups detailed below have been stable for some time. The phylum is divided into three classes:-

- Phylactolaemata, a small and distinct group, very different from all other bryozoans. Zooids are essentially cylindrical, with a horseshoe shaped lophophore with an epistome. The body wall is uncalcified, and the coelom is continuous from one zooid to the next. New zooids arise by replication of polypides which occurs prior to the differentiation of the cystid. Polymorphism is completely absent. All species are only found in freshwater.
- Stenolaemata, a much larger group, although predominantly fossil. Zooids are cylindrical, with a calcified body wall and, in extant forms, a circular lophophore without an epistome. All zooids have their own discrete coelom and new zooids arise by the division of septa, with inception of the polypide, preceding the differentiation of the cystid. There is limited occurrence of polymorphism. All species are marine. The class is divided into five orders, only one of which, the Cyclostomatida, contains extant species.
- Gymnolaemata, by far the largest group of bryozoans, composed of two very different orders:-
    - In the first, and very much the smaller group, Ctenostomatida, the zooids are squat or cylindrical, gelatinous or membranous, but always with an uncalcified body wall. The lophophore is circular without an epistome and the orifice is often terminal and closed by a pleated collar. The coeloms of adjacent zooids are separate and new zooids are produced by deposition of septa, with cystid formation occurring

before polypide inception. Polymorphism is of limited occurrence. The majority of species are marine.

- The second suborder, Cheilostomatida, contains the vast majority of all extant bryozoans. Zooids are generally box-shaped and have calcified body walls. The lophophore is circular without an epistome and the orifice is invariably closed by an operculum. Coeloms of adjacent zooids are separate, new zooids are produced by the deposition of septa, with cystid formation occurring before polypide inception. Polymorphism is very highly developed, in terms of frequency of occurrence and the variety of forms. The vast majority of species are marine.

1.6.3 Reproduction

All bryozoans are hermaphrodites and within the cheilostomes autozooids may be dioecious or monoecious, frequently protandrous but sometimes protogynous. In dioecious species male and female autozooids may exhibit different morphologies. Sperm are released through the tips of the tentacles. Ripe eggs are released into the coelom and in oviparous species released via a tube, the intertentacular organ, a modified coelomopore. In brooding species, sperm are presumed to enter the maternal zooid via the coelomopore and fertilization occurs precociously within the zooid (egg activation may be much delayed) (Temkin, 1996), and embryos are brooded in a variety of structures but most commonly in ovicells. Their spatial disposition relative to the maternal and distal zooid exhibits variation and a variety of different types have been distinguished. Ovicells do not always develop in the same way (Section 1.6.4.5). The eggs of oviparous species develop into often-bivalved planktotrophic larvae (cyphonautes) which may live in the plankton for several months, and whose power of dispersal is therefore considerable. Brooding species release large lecithotrophic larvae, the vast majority of which are coronate, but a few are shelled (Zimmer and Woollacott, 1977). All settle within a few hours, and have, therefore, very limited powers of dispersal.

It is an oversimplification to relate actual dispersal to the length of larval life. Porter et al. (2002) looked at two species of *Alcyonidium* one of which has planktotrophic

and one lecithotrophic larvae. The former, *A. mytili*, colonises hard surfaces whilst the latter, *A. gelatinosum*, is found on algal substrata. Whilst genetic variation within a population was greater in the former the difference was less than expected. Although *A. mytili* produces cyphonautes type larvae (Cadman and Ryland, 1996) the length of the free-swimming phase is not known. It is also possible that in the populations investigated, in estuarine or ria environments, larvae are retained within the system by tides which essentially move water up and down the channel (Young and Chia, 1987). Watts (1997) also found a lower level of gene flow between populations than expected in *Electra pilosa* and *Membranipora membranacea*, two species with long lived planktotrophic larvae (Atkins, 1955). It is also possible that epiphytic species such as *A. gelatinosum* could achieve dispersal by rafting on detached algal fronds (Porter et al., 2002).

Oviparity and planktotrophic larvae are considered the primitive condition. Larvae search for suitable substrata, adhere to this by eversion of an adhesive sac, and metamorphose into the first zooid(s) of a colony, the ancestrula. This is generally a single zooid but in some cases two or more.

Within the ctenostomes dioecious autozooids are unknown (Hayward, 1985). In brooding species embryos are usually held within the tentacule sheath. A minority of species are oviparous, producing small planktotrophic larvae rather similar to the cyphonautes of cheilostomes. The brooding species exhibit variation in the brooding process but all produce coronate lecithotrophic larvae.

Within the cyclostomes eggs develop only in dimorphic female gonozooids, and it is possible that all cyclostome autozooids are dioecious (Hayward and Ryland, 1985). Internal fertilization results in a primary embryo, from which blastomeres detach, continue cleavage to form secondary embryos, which may in turn fragment to form tertiary embryos. Polyembryony in animals generally is rare, and Ryland (1996) has suggested that it occurs here perhaps in relation to a shortage of sperm. Polyembryony has resulted in the production of brood chambers, highly modified zooids, gonozooids, containing a number of genetically identical embryos.

Gonozooids vary in morphology and perhaps ontogeny (Hayward and Ryland, 1985).

Astogeny, the process by which a colony is formed by repeated budding, initially from the ancestrula, is central to the biology of bryozoans and their colonial nature. Because I wish to discuss this sequentially with colony form, the spatial arrangement of zooids within colonies, and variations in the degree of colonial integration, I shall delay treatment of astogeny until I have considered polymorphism.

1.6.4 Polymorphism

1.6.4.1 INTRODUCTION

"Polymorphism is the particular mechanism evolved by colonial organisms for maximising efficiency in the division of labour, comparable to the evolution of organ systems in solitary organisms" (Abbott, 1973).

For all colonial marine invertebrates the definition of polymorphism as "discontinuous variation in the morphology of zooids arising at the same astogenetic level" (Boardman and Cheetham, 1973) is generally accepted. The idea that some polymorphs, intercalated in the normal budding pattern and capable of budding further zooids, such as the vicarious avicularia in *Steginoporella*, represent modified autozooids was proposed by Harmer (1900). In some species of this genus, in addition to the 'ordinary' A-zooids, there exist B-zooids which have an augmented operculum and opercular muscles. In certain other congeneric species there are vicarious avicularia, but in no species do B-zooids and avicularia occur in the same species (Silén, 1938; Banta, 1973). Banta reasoned that if vicarious avicularia developed from polymorphic autozooids, for adventitious avicularia to develop from vicarious forms required that the avicularium buds were transferred from the primogenial to an adventitious position; and that this was must have occurred in the development of frontal budding of autozooids.

There is, however, no universal agreement on the status of all adventitious polymorphs developing from an autozooid budding site which does not normally give rise to another autozooid. Such polymorphs generally do not possess the ability to bud a further zooid and their appearance is not unlike an ornamentation of a zooid's body wall. A polymorphic zooid is distinguished from the latter by the presence of a pore-plate separating the two body cavities (Cheetham and Cook, 1983). For some polymorphs, such as spines, the area of origin of which is very limited, this may not be readily apparent (Silén, 1977).

The idea that some, if not all, polymorphic zooids are modified or reduced autozooids was investigated by Cheetham (1973) using principal component analysis. He concluded that, for the cheilostomes *Poricellaria* and *Vincularia*, evolutionary trends in morphologic variation were consistent with gradual transformations between monomorphic and polymorphic zooecia, whether this was progressive or retrogressive.

Within the Bryozoa "polymorphism is represented to such an extent and takes such a multitude of often very specific expressions, that great biological importance must be ascribed to it" (Silén, 1977). However, although widespread, its level of occurrence varies considerably between groups at all taxonomic levels within the phylum, and also often within them. Some of this variation is a direct result of the different morphologies of the autozooids of the higher taxonomic groups, and the fact that polymorphic zooids arise by heterochrony. They are variations on a theme, and as such, the possibilities are circumscribed by the characteristics of that theme. Avicularia and vibracula owe their existence to the presence, in the vast majority of cheilostomes species, of a hinged operculum which closes the aperture after lophophore retraction. They do not occur therefore, outside the Cheilostomatida. Interestingly, a group of Cretaceous and Palaeocene cyclostomes, meliceritítids, did have operculate zooids, and were highly polymorphic with avicularia-like eleozooids (Taylor, 1985).

Polymorphism is absent in the Phylactolaemata, is not well developed in the Cyclostomatida or the Ctenostomatida, and is extensively developed, if unevenly distributed, in the Cheilostomatida.

1.6.4.2 POLYMORPH MORPHOLOGIES AND FUNCTIONS

The complete range of polymorphs has been classified in two different ways, neither of which is entirely satisfactory, essentially related to morphology or function. A major problem in using function as a basis for classification is that it is not always known, and the function of a particular polymorph has often been assumed on the basis of the function of another polymorph of similar morphology. This is a dubious practice as it is known that certain polymorphs of similar morphology fulfil different functions in different species. It also demonstrates that using morphology alone as a basis for classification is also not without its problems.

Silén (1977) classified polymorphs according to known or assumed functions and distinguished those functioning in:-

- active defence
- passive defence
- cleaning
- colony strengthening
- colony support or attachment
- survival of unfavourable conditions
- interzooidal connections
- sexual reproduction

Although there are a great variety of polymorphs, the vast majority are variations on only two basic types. Polymorphs can be distinguished by the nature of the modifications their development has undergone rather than by their morphology alone.

- Many polymorphic zooids are forms of kenozooid, empty zooids, which essentially consist of a body wall, which may vary enormously in shape and

extent, a coelom and a funiculus connecting them to the rest of the colony. Polymorphs of this general type, are to be found in all taxa of Bryozoa which exhibit polymorphism, but are most varied and numerous within the Cheilostomatida.

- A second frequently found type, within the Cheilostomatida, is that in which the operculum of the autozooid is hyper-developed into a mandible (avicularium), or a seta (vibraculum). In the vast majority of cases, the polypide is non existent or vestigial, and the coelom is largely occupied by the enhanced musculature necessary to power the mandible or seta.

Kenozooids exist in a wide variety of morphologies and locations and fulfil a variety of functions:-

- They form simple interzooidal connections, pore-plates, between cheilostome zooids (Hyman, 1959; Silén, 1944, 1977).
- They may function in colony structure, notably as stolons in the ctenostomes where they often form a framework facilitating the spatial arrangement of the autozooids within a colony.
- They may, particularly in encrusting species where irregularities in the substrata result in spaces too small for an autozooid to develop, act as 'packing' ensuring the continuity of the colony.
- They may function as stylozooids (Silén, 1977) in elevating feeding autozooids into the water column. A series of turgid stylozooids form a peduncle elevating the feeding capitulum of autozooids above the substratum in *Metalocyanidium gautieri* (Hayward, 1985). In *Semikinetoskias* the colony is supported on a single long stylozooid. (Silén, 1941)
- They may act to strengthen colonial structure. In frondose forms of the Flustridae, kenozooids with particularly thick walls may occur in a proximally broadening band along the frond edge (Silén, 1977). In *Securiflustra securifrons* rows of kenozooids at frond edges are also responsible for any increase in zooid rows due to bifurcations (Hayward and Ryland, 1998).
- In encrusting species, in the form of stolons, they may prevent overgrowth. Such stolons in *Membranipora membranacea*, produced to obstruct the

growth of conspecifics, grow very rapidly, and may become six times longer than an autozooid (Harvell, 1994).

- In non-encrusting species they function, in the form of rhizoids, for attaching the colony to the substratum, or one part of it to another. They are long tubular structures and, for the former, show a positively geotropic form of growth, with a means of attachment to the substratum. This varies in nature and in some species of Candidae may develop, according to the substrate encountered, a gripping holdfast, or a penetrating grapnel with recurved hooks. Rhizoids which connect two branches of the same colony grow to a pore plate of the destination branch (Silén, 1941).
- Finally, as spinozooids, they function in passive defence. They frequently form a vertical barrier around the distal end of the frontal membrane. In the Candidae one spine may develop into an extensive structure, the scutum, and overarch the frontal membrane protecting it to a greater or lesser extent. In the cribrimorphs spines grow horizontally over the membrane and partially fuse together forming a shield.

All kenozooids are without moving parts and have no behaviour which can be observed; their function can therefore only be deduced from their form and location. Generally this seems beyond debate.

Avicularia occur very frequently, rarely as vicarious forms but usually adventitiously. They exist in a great variety of morphologies and sizes, and the fact that two or more different forms may be present in a single species suggests that they do not always perform identical functions. Because of the rapid manner in which a mandible may be closed, and the reinforced character of the rostrum to withstand its impact, avicularia are assumed to have a grasping function related to defence (Silén, 1977). This has rarely been observed except in respect of the large pedunculate avicularia of genera such as *Bugula*. Kaufmann (1971) found they were effective against tube-building crustaceans <4 mm long and <0.05 mm in diameter. Wyer and King (1973) found no evidence that the avicularia of *Bugula* or *Flustra* were any defence against pycnogonids. The fact that in some forms of avicularia the mandible may project beyond the rostrum, and occur in a variety of shapes, suggests that not all function as

graspers (Silén, 1977). The spatulate mandibles of often-large sporadically occurring avicularia have been associated with the inferred function of cleaning by the generation of local water currents (Cook, 1985). Whilst the pedunculate avicularia of the Bugulidae have movement, the vast majority are immobile. They occur in a variety of positions relative to their autozooid of origin.

The function of the vast majority of avicularia remains a matter of speculation. They occur in the majority of cheilostome species, they may be very numerous within a colony, and also exist in a variety of forms. Many are very small, and they are not always sited in obviously defensive locations. Most extremely, the occurrence in certain species of *Menipea* of avicularia apparently within the body cavity (Levinsen, 1909; Harmer, 1923) is very difficult to explain in functional terms. Beyond the pedunculate avicularia referred to above no defensive function, or any other, has been demonstrated for avicularia by Winston (1984). She made a number of lengthy observations, under laboratory conditions, and most revealed no behaviour at all! Although these observations were made very quickly on introducing the colonies into the laboratory, one does not know in what respects, and to what degree, their behaviour may have been affected by the environmental changes they experienced.

A variety of other possible functions of avicularia have been suggested, e.g. nutrient storage (Cook, 1979) and respiration (Waters, 1889), but there is little evidence to support these (Winston, 1984). Chemical defence was put forward by Lutaud (1969), who suggested that they could be centres of chemical production. Lidgard (1981) has shown that strong water currents, containing debris of varying origin, exist just above the surface of the colony, having passed through the lophophores and exited via chimneys or at the edge of the colony. Prominent frontal avicularia, Winston (1984) suggested could act as current baffles/trash chutes. None of the above explains why avicularia should have a mandible.

Vibracula occur much less frequently than avicularia, being especially characteristic of two groups with very different colony form, the arborescent Candidae and certain lunulitiform genera. They exist uncommonly as vicarious forms, the vast majority being adventitious. They are less variable in form than avicularia. Vibracula are

characterised by a generally very long and mobile seta and are generally assumed to perform a cleaning function, especially in arborescent forms, removing detritus and perhaps also preventing organisms settling on the colony surface. Cook (1985) observed of the setae of *Scrupocellaria*, that they "are capable of movement in two planes and three directions.....and the movements are then reversed". The movements of the setae are not coordinated within the colony (Silén, 1950). Vibracula perform a very different function in free-living lunulitiform species, providing colony support and the ability to right overturned colonies (Cook, 1963).

Winston (1984) in respect of avicularia, having had little success in provoking any response from them, suggested that not all polymorphs actually fulfil a function within the colony, and that perhaps they have not been selected for, they just occur as result of the budding process. She went on to say "the adaptionist orientation of most bryozoologists over the last 100 years....has produced little evidence to explain the occurrence of avicularia in bryozoans. Perhaps the basis of the polymorphism is not ecological but developmental". Given that many polymorphs fairly obviously do perform useful functions (see Section 1.6.4.3, below) this would seem an over reaction.

There are other polymorphs with a more limited occurrence.

Within the Cyclostomatida the nanozooids of the Diastoporidae (described by Borg, 1926) resemble autozooids but are considerably smaller, have no gut, and a reduced polypide with a single long tentacle. This is normally motionless but periodically sweeps from its proximal orientation to a distal one and back again. Much less frequently it moves in a circular motion until it regains its original position (Silén and Harmelin, 1974). Nanozooids occur in a strict one to one ratio with autozooids (Hayward and Ryland, 1985). In *Diplosolen obelia*, Silén and Harmelin found them so sited that collectively they swept the entire frontal surface of the colony. They presumably remove detritus and discourage settlement.

Spinozooids in some crisiids are not single kenozooids, as in the cheilostomes, but are composed of a series of such kenozooids (Hayward and Ryland, 1985).

The cyclostomatous gonozooids are budded within the normal autozooid sequence but the body wall subsequently expands to enclose an often much larger volume, to house the numerous embryos which result from polyembryony. Their form, and that of their modified orifice, varies, and is often species specific. It is possible that some brood chambers are not actual zooid homologues. Borg (1926) regarded those of the Lichenoporidae as constituting a part of the zoarial coelomic cavity and thus as zoarial brood chambers. In many species however, they are highly modified zooids, the body wall of which subsequently expands to form a voluminous chamber (Hayward and Ryland, 1985).

The above has been restricted to polymorphic heterozooids, and one example of a polymorphic autozooid is necessary. In encrusting species the normal feeding water current takes water into the tentacle crown, toward the mouth, and then between the tentacles towards the colony surface. Where this is extensive, groups of zooids with modified asymmetrical lophophores protruded at an angle may form 'chimneys', which form exhalent water outlets (Cook, 1977; Cook and Chimonides, 1980).

In some dioecious species there is sexual dimorphism which may involve differences in zooid size and/or morphology, and/or the nature of the lophophore; e.g. *Celleporella hyalina* (Hayward and Ryland, 1999) and *Alcyonidium nodosum* (Ryland, 2001).

1.6.4.3 GENERAL REMARKS REGARDING POLYMORPHIC HETEROZOOIDS

The fundamental questions regarding polymorphic heterozooids are why they are present, are as they are, in the number that they are, and where they are. Variations in the occurrence of polymorphs in relation to autozooids, internodes, and the colony as a whole could throw some light on their function and/or their importance in terms of the organisation of the complete colony. All heterozooidal polymorphs are, by definition, a nutritional drain on the colony. If the colony is the unit upon which natural selection acts one would expect polymorph production, in terms of number, morphology, size and siting, to be optimised, maximising the benefits relative to their costs.

There is much evidence given in Section 1.6.4.2 that many polymorphic heterozooids perform particular functions in a sophisticated division of labour. The assumed functions, on the basis of their position within the colony or in relation to a constituent part of it, of virtually all kenozooids would seem perfectly reasonable and beyond debate. Kenozooids connecting, strengthening, spacing, reinforcing, or elevating autozooids, surely do just that. Spines protect vulnerable areas and rhizoids attach non-encrusting species to the substratum. Within the Cyclostomatida the morphology and spacing of nanozooids results in the entire frontal surface of the colony being subject to their sweeping tentacles. The large gonozooids of the group are able to contain the multiple embryos resulting from polyembryony.

Nevertheless there are two major problems.

Firstly, although the assumed function of many polymorphs is probably correct, their occurrence might be expected to be more frequent and more regular. The widespread variation of occurrence of polymorphs within a colony, a species, and indeed at all taxonomic levels is difficult to understand. These irregularities are not easily explained in functional terms. Species, and indeed colonies, may differ in their ability to produce a particular polymorph, and variable environmental conditions may cause the need for it to vary. Further, genetic variation may affect the threshold level at which colonies respond to these. Intrinsic and extrinsic factors in polymorph initiation interact, and given that compromises are probably necessary between competing requirements, it would seem likely that some kind of hierarchy operates. In certain instances the space available for a zooid determines whether an autozooid or a particular polymorph is produced. The production, for example of an avicularium rather than an autozooid or a kenozooid, purely in response to the size of the available space is difficult to explain in functional terms. It would seem to result simply from the astogenetic 'rules' of the species 'deciding' which zooid type should be produced.

Secondly, that the function of the vast majority of avicularia, and to a lesser extent some of the vibracula, is far from clear. It was in respect of the former that Winston (1984) failing to find any evidence supporting the conventional view of a defensive

function, suggested that perhaps either they performed some completely different function, or that they did not have a division of labour type function at all!

1.6.4.4 CUES INDUCING POLYMORPHS IN THE BRYOZOA

For constitutive polymorphs that occur absolutely constantly or predictably, no intrinsic or extrinsic environmental cues are involved in their production. For all other polymorphs, "the expression of polymorphism in bryozoans appears to be hierarchical with both intrinsic and extrinsic cues operating" (Harvell, 1994). Silén (1977) expressed a similar view, differentiating the colonial from the external environment.

A single factor which can be influential in determining whether a zooid develops into an autozooid or some form of polymorph is simply its size. In certain encrusting species, whether a zooid develops into an autozooid, an avicularium or a kenozooid, appears to depend purely on the size of the zooecium. In *Thalamoporella,* zooids below a certain size became avicularia, and below a smaller size still, developed into kenozooids (Silén, 1938; Powell and Cook, 1966). In this case it appears that a single intrinsic cue, zooid size, is involved. That zooid size does not always act alone is apparent from the case of the stolons produced at colony edges in the presence of conspecifics by *Membranipora membranacea.* Such stolons may be four or five times the length of normal autozooids, but are kenozooids without polypides. The species also produces kenozooids when zooid size is below a certain threshold, and autozooids with two functioning polypides in extra large zooecia. In autozooid development, polypide inception occurs after calcification of the body wall has begun. Stolons remain uncalcified and it is conceivable that polypide formation and calcification are cued by the same developmental environment, or that polypide inception requires the cue of calcification (Harvell, 1994).

A second factor, which may be very important in respect of whether or not a polymorph develops, is the age of the autozooid from which it develops. The spines which *Membranipora membranacea* may produce, in response to the presence of one of its nudibranch predators *Doridella steinbergae* or *Corambe pacifica* (Yoshioka, 1982) develop from spine chambers which are always present. Such spines develop

only from the spine chambers of ontogenetically young autozooids around the growing edge. Zooid age alone, in this case, appears to determine the ability to respond to an external inducer (Harvell, 1991). This case clearly involves an intrinsic cue, defining when an extrinsic cue should result in the production of the polymorph. The interaction of the two results in only those young autozooids around the periphery of the colony producing defensive spines. These do not act as a barrier protecting the complete colony; the unprotected centre is attacked but the spined peripheral zooids suffer much-reduced predation and are generally able to regenerate the central region (Harvell, 1984).

In general it would seem arguable that the production of those polymorphs (which do not occur constantly or regularly because they are constitutive) whose function is in relation to the integrity of the colony, packing or strengthening kenozooids, would be essentially controlled by intrinsic cues. Conversely, those polymorphs whose function is directly related to the external environment, one would expect to be controlled more by external cues. As shown above, however, internal and external cues may interact.

1.6.4.5 OVICELLS

The majority of cheilostomes brood their larvae and in the majority of species this occurs in essentially globular ovicells. An ovicell is here defined, following Nielsen (1985) as "the hooded structure exclusive of closing structures such as the ooecial vesicle and operculum". The ovicell wall consists of an outer layer, ectooecium, generally calcified, and an inner layer, endooecium, frequently membranous (Hayward and Ryland, 1998). Ovicell structure has been poorly understood, and the literature is often ambiguous and contradictory (Santagata and Banta, 1996).

Woollacott and Zimmer (1972) looking at the origin and structure of the ovicell of *Bugula neritina* showed that it formed from the zooid distal to the maternal zooid, and that it was separated from its zooid of origin by a pore plate; it was a kenozooid. Nielsen (1981) concluded that with few exceptions such as *Thalamoporella* and *Scruparia*, cheilostome ovicells were probably formed from the zooid distal to the

maternal zooid. In a further study involving four cellularine species (Nielsen, 1985) distinguished between three species of the Candidae in which the "ovicells are integral parts of the distal zooid"; from *Bugula pacifica* in which "the connection with the autozooid becomes very narrow and is probably a pore connection". In many cheilostomes, including some species of *Scrupocellaria*, the ovicell primordium is bilobate (Levinsen, 1909), consistent with derivation from a pair of modified spines, a hypothesis initially put forward by Harmer (1902), (Santagata and Banta, 1996).

Ovicells derived from a single primordium are kenozooidal polymorphs, whilst those of a bispinose origin are perhaps best considered as polymorph composites. The situation is made more complex when the gymnocyst of the distal zooid forms the floor of the ovicell. I have, therefore, in the central chapters of this study, concerned with species of *Scrupocellaria* and *Tricellaria*, referred to 'polymorphs' and 'ovicells'. Ovicell production by one zooid for the embryo of another; and ovicells constituted of more than one polymorph; are both indicative of a high level of colonial co-ordination.

1.6.5 Budding and astogeny

1.6.5.1 BUDDING

All zooids beyond the ancestrula arise by budding, and variations in the way this occurs underlie the resulting spatial arrangement of zooids within the colony. Within the encrusting Cheilostomatida, three main types of budding (and within these, sub-types) were distinguished by Lidgard (1985).

- Intrazooidal, in which a new zooid develops from a pre-existing space in an existing zooid.
- Zooidal, in which a new coelomic space is created, which is then separated from the parent zooid by an interior wall.
- Multizooidal, as zooidal but the new space created is much longer than a single zooid and is partitioned by a series of interior walls.

Colonies exhibiting intrazooidal or zooidal budding may develop in linear series or into sheets. In coalescent multiserial (Lidgard, 1985) each zooid develops not from a single progenitor but from the fusion of several. In discrete multiserial colonies, the zooids of each series are separated by interior and the series by exterior walls; these are distinguished from compound linear in which some series are separated by interior walls. In compound non-linear colonies all zooids are separated by interior walls (Lidgard, 1985).

1.6.5.2 ASTOGENY

Astogeny was defined very succinctly by Boardman, Cheetham and Cook (1969) as "the course of post larval development of a colony". It is the process of colony growth by budding following the original settlement and metamorphosis of the larva. It is a major determinant of the arrangement of all zooids, autozooids and heterozooids, within a colony. There is much variation in the degree to which the astogenetic 'rules' determine this, and the colony structure and form which results.

All bryozoans exhibit some characteristic pattern of growth, both in respect of autozooids and heterozooids, as a result of the characteristic astogenetic pattern of the species. Those species in which astogenetic control is not well developed are more susceptible to the influence of environmental factors. In encrusting species which achieve no overall structure or form, and the extent of whose growth is much determined by the extent and nature of their substrata, astogenetic patterns are generally loose. Nevertheless even encrusting species have characteristic budding patterns. *Membranipora membranacea* colonies always advance on a broad front, whilst those of *Electra pilosa,* advance on a number of narrow fronts but may subsequently 'backfill' the spaces between them. Species such as *Flustra foliacea,* commence growth as an encrusting sheet, which develops into a bilaminar erect frond, with two equally extensive surfaces of autozooids growing back to back, and which subsequently divides from time to time, to form an extensive, bilaminate lobate structure (Stebbing, 1971; Silén, 1981). Lace corals (Phidoloporidae) develop three-dimensional forms, by folding and enrolling reticulate bilaminate sheets, with a frontal surface of autozooids and polymorphs, and a basal surface of kenozooids.

Such structured colonies involve a high level of colonial organization and integration (Hayward and Ryland, 1998).

Arborescent bryozoans generally have a more or less determinate structure and form, evidence of a tightly controlled astogenetic pattern. Internodes (branches) in many species are biserial, whilst they have the potential to form new lateral rows in a number limited only by available space (Silén, 1977). The form of such internodes requires the suppression of rows of autozooids which could develop. Silén went on to say that such autozooid rows are either directly totally suppressed or suppressed via the production of dwarf zooids, adventitious polymorphs. I find the latter difficult to square with his assertion of the great biological importance of polymorphism. The production of numerous heterozooids would seem a very extravagant way to bring about the suppression of a row of autozooids. Further, when such dwarf zooids are not produced why do autozooids not develop? The autozooids within the internodes of such species occur in several different forms related to their position within the internode. In the arborescent cellularine species of this study, the simple and branched spines associated with all autozooids except the ancestrula are similarly arranged in relation to the position of their autozooid within the internode. Astogenetic pattern is ubiquitous.

Related to astogeny is the phenomenon of astogenetic change. There may well be gradual changes in the morphology in the early generations of autozooids, defining a zone of astogenetic change, which is followed by a much more extensive zone of astogenetic repetition in which autozooids essentially have the same morphology. Patterns may be barely discernible or more complex.

Although astogenetic change is a well-known phenomenon and is usually very limited in extent and gradual in its progression, this is not always the case. *Corbulipora tubulifera,* described by Bock and Cook (1994), exhibited three very different autozooid morphologies. Three previously described species in three different genera, and two different families, were found to be astogenetic and ontogenetic phases of the same taxon!

1.6.6 Colony form

Bryozoan colonies exist in a number, but not unlimited variety, of forms, which may be grouped into four types.

1.6.6.1 ENCRUSTING

The great majority of species are encrusting and form unilaminar colonies, varying considerably in shape and extent, on a great variety of substrates, inorganic and organic. In a limited number of species growth occurs as a number of linear runners, but more commonly the colony covers an area completely. In some species new zooid layers develop above the initial layer, and in others the colony develops a nodular form.

1.6.6.2 ERECT

A sizeable minority of species adopt a variety of upright forms. The majority of free-standing upright species possess specialised attachment zooids, rhizoids, by which they are attached to the substrata. In a minority of species there is an initial encrusting phase before erect growth begins. In some 'rooted' species living on soft sediments the rhizoids attach to sand grains or similar very small substrate. Such species invariably have a series of kenozooids serving to raise the feeding autozooids clear of the sediment surface.

The vast majority of erect forms grow distally and laterally by the production of new branches or lobes, and the manner in which this occurs gives rise to a variety of colony morphologies, the nature and form of the upright component varying considerably. In the first instance a distinction can be made between flexible and rigidly erect forms. Flexibility may be achieved in a variety of ways. It may result from an absence of calcification in ctenostomes such as *Alcyonidium*, light calcification in cheilostomes such as *Bugula*, or the possession of non-calcified joints, nodes, between calcified branches in arborescent bryozoans such as *Crisia*, *Cellaria,* and cellularines such as *Scrupocellaria.* Within the cheilostomes, erect colonies may be unilaminate such as *Scrupocellaria* or bilaminate as in *Flustra*

foliacea. Autozooids may be arranged in narrow branches as in the former or broad lobes as in the latter. In ctenostomes such as *Alcyonidium* and cheilostomes such as *Cellaria* autozooids are arranged all round an essentially circular 'stem'. Rigidly erect forms may also be unilaminate, with narrow, or bilaminate, with much wider or cylindrical, branches. In both, in the absence of flexibility, the colony may be strengthened by branches being linked or anastomosing, and in bilaminate adeonifom species by heavier calcification.

1.6.6.3 FREE LIVING

A few species exist as free-living colonies with the form of a small inverted saucer, and are held above the substrate by very long vibracular setae. Other species are sometimes found free living, although they have, in all probability, adhered initially to a limited substratum, have subsequently completely surrounded it, and the aggregation then became detached.

1.6.6.4 BORING

Another very small number of species inhabit a substrate by boring into it and generally form linear runners between two different layers within it (Pohowsky, 1978). Similar in form are species which live between different layers of tubes secreted by polychaetes.

The above attempts to classify a diverse assemblage into a limited number of basic types. Although there are variations within them, bryozoan colony form is essentially conservative, with very similar forms persisting over evolutionary time (McKinney and Jackson, 1989).

1.6.7 Bryozoan colonies vary in the degree of their colonial integration

The diversity in form of Bryozoan colonies is accompanied by a similar diversity in the level of colonial integration. The extensiveness of the fossil record and the difficulties associated with observing live material, at the scale necessary to discern

colony-wide patterns, has resulted in theoretical considerations based on hard part morphology being the dominant approach (Cook, 1979).

Variations in the degree of colonial integration and the elements contributing to it were extensively reviewed by Boardman and Cheetham (1973). They inferred various degrees of colony dominance from variations in respect of six morphological characteristics:-

- The nature of zooid walls.
- The nature of soft-tissue inter-zooidal connections.
- The presence/absence of extrazooidal growth.
- The complexity of the astogenetic process.
- The extent of morphological differences between polymorphic zooids.
- The positioning of polymorphs within the colony.

I shall confine myself to their discussion regarding the Cheilostomatida.

For colonies composed of a number of individual zooids, it is possible to envisage theoretically, in terms of their degree of integration, a continuum of possibilities. At one extreme the zooids have complete independence, whilst at the other colony control or domination is total. In reality the continuum is not likely to reach either extremity. The transition from zooid independence to colony control is the result of increasing integration of zooids and perhaps the development of extrazooidal parts. Whilst autozooids are very small, and relatively constant in size and shape, a considerable range of colony size and form are achieved via variations in the number of autozooids, and their spatial arrangement in relation to one another. In the vast majority of cheilostomes in addition to the autozooids, there exist a variety of polymorphs, generally heterozooids, the morphology of which are modified, often very considerably.

Morphological variation of zooids within colonies is not restricted to polymorphic individuals, and Boardman et al. (1969) distinguished four sources and types of such variation:-

- Ontogenetic variation.
- Astogenetic variation.
- Polymorphism
- Variations due to environmental factors

For each of the six morphological characteristics listed above, Boardman and Cheetham (1973) described a series of states, which they arranged in order of increased integration:-

- The distinction between exterior and interior body walls (Silén, 1944; Banta, 1969) is important in determining degrees of colonial integration. The former – in that they do have the ability to separate a zooid from the external environment – express zooid autonomy, whilst the latter – which do not possess this ability – reflect colony dominance.
- The presence of interzooidal connections by soft tissue is not a feature of solitary animals, and clearly expresses colony dominance.
- The presence of extrazooidal parts, which are not part of any zooid, is indicative of colonial control.
- The presence/absence, and the extent, of generational differences in zooid morphology, astogenetic change, is a characteristic indicative of the degree of colony dominance.
- The range of morphological variation between polymorphic zooids provides some measure of the degree of colony dominance.
- The extent of the structural dependence of polymorphic zooids on another zooid, and the positioning of polymorphs within a colony, random, regular, grouped, are both indicators of the degree of colony dominance.

For each of the above, a series of four or five actual or postulated states were described, ranging from zooid autonomy to colony domination. The theoretical framework, described above, was applied to 16 cheilostome species or groups of species.

Firstly, zooid walls, the nature of which is closely related to the mode of budding. In the majority of cheilostomes zooids are produced in linear series (Boardman and

Cheetham, 1969) each of which is bounded frontally, laterally and basally by exterior walls, whilst the zooids within each series are separated by interior walls. The higher the proportion of interior walls the greater the level of colony dominance.

Secondly, soft tissue connections between zooids. Whist some minor variations exist, within the order there is little variation.

Thirdly, the presence or absence of extrazooidal parts. In the majority of the cheilostomes none are present, but in a minority, e.g. *Cupuladria*, some such parts are well developed. Such development is indicative of well-developed colonial integration.

Fourthly, the presence/absence and complexity of zones of astogenetic change. In the majority of cheilostomes there is a single, proximal zone of astogenetic change, but in a minority of species more than one such zone exists. The greater the complexity of this pattern the greater the level of colony dominance.

Fifthly, the extent of morphological variation between polymorphs. Polymorphism is characteristic of the cheilostomes, although variable in extent between taxonomic groups at all levels. The greater the range of morphological variation the higher the level of colonial integration.

Sixthly, the degree of structural independence of polymorphs, and the regularity or otherwise of their location. Polymorphs intercalated in the budding pattern, vicarious, are considered more structurally independent than those which are not so budded, adventitious, and are structurally dependent on the autozooid which produced them. Vicarious polymorphs which occur, apparently randomly within the colony are deemed to be more independent than those whose siting is regular and or predictable, and therefore assumed to be under colonial control. (Interestingly, within this last series, the regularity or otherwise of adventitious polymorphs, was not considered).

The delineation of six relevant characteristics does not, in itself, indicate their relative importance in regard to colonial integration The regularity of the actual

spatial arrangement of autozooids within the colony, a parameter difficult to quantify, was not considered, but is surely relevant.

A range of levels of integration occur within the cheilostomes, in four of the six characteristics (the level of interzooidal communication and the range of morphological variation between polymorphs show little variation within the order). There appears to be no simple progression from less to more integrated types. Certain groups may be highly integrated in certain respects but low in others, whilst other groups exhibit a very different mix. The observed pattern suggests that there is more than one adaptive trend within the cheilostomes.

Beklemishev (1969) considered that adaptive trends should be related to characteristics which " intensify the individuality of the colony". Boardman and Cheetham (1973) felt that three of the trends identified by Beklemishev were particularly important in respect of the evolution of the cheilostomes:-

- Structural integration, through the development of structures shared by zooids, or to a lesser degree, external to them.
- Physiological integration by increased communication between zooids. (Although within the group there appeared little variation).
- Increased functional integration, via specialised polymorphic zooids, and often their joint activity.

Boardman and Cheetham (1973) felt that increased regularity of budding and astogenetic complexity, were in part, related to these.

With regard to structural integration, it is notable, within the species studied, that extrazooidal parts were developed in those species whose vertical walls were largely interior, e.g. *Cupuladria,* and not developed in the majority of species in which exterior vertical walls were also present.

With regard to physiological integration, essentially dependent on the degree of communication between zooids, there was no evidence of evolutionary development.

With regard to functional integration, increased complexity of astogenetic changes; the number, variety, and precision of siting of polymorphs within the colony, could all be argued as increasing its functional integration.

The somewhat artificial division of integration into these component parts did facilitate some generalisations relating the character of the integrative state to particular colony forms.

For non-encrusting forms, generally achieving a definite colony form, some degree of colonial integration is clearly necessary. The most structurally integrated species were rigidly erect or free-living forms, for which the danger of breakage was greatest. The most functionally integrated species were flexible, jointed, erect species. Jointing would appear to require some colonial control of branching, via budding, and the more regular siting and/or grouping of polymorphs. Encrusting forms generally exhibited a range of generally low-level structural and functional integration, and some ancestral species with a very low level of integration, still exist. Encrusting species have no colony form to achieve, and their size is much related to that of the available substrate.

The above theoretical considerations relate levels of structural and functional integration, in some degree, to variations in colony form discussed in Section 1.6.6.

A complementary, but less-utilised approach, involving the observation of live material, together with inferences from hard part morphology, may be necessary to reveal certain aspects of colonial integration, especially where there is a behavioural element. Cook (1979) envisaged a gradient of colonial integration, from colonies in which zooids function almost as an aggregation of solitary animals (e.g. *Aetea*) to others in which colony control of constituent zooids is considerable (e.g. *Cupuladria*). In *Aetea* the budding pattern is uniserial, there is no colony form, the majority of zooid walls are in contact with the environment, zooids are monomorphic, are in contact via a single pore, and each feeds separately. In the highly integrated colonies of *Cupuladria*, the discoid colonies are free-living, have a regular budding pattern and colony form, and only a small fraction of zooid walls are

in contact with the environment. Zooids communicate through several pores, both with each other and with a basal colony-wide coelom (Håkansson, 1973). Some 50% of zooids are heteromorphs and require nutrition from autozooids. That the peripheral setae may function simultaneously, suggests that behaviour may also be colony controlled (Cook, 1963).

The role played by groups of autozooidal polymorphs with asymmetrical tentacle crowns in relation to a colony-wide arrangement of excurrent 'chimneys' facilitating the disposal of already filtered water (Cook, 1977; Cook and Chimonides, 1980) was referred to in Section 1.6.4.2. These may involve differences in behaviour and/or morphology, and may be centred on a non-feeding zooid. The production and maintenance of such groups indicates considerable colonial control of zooid morphology and behaviour (Cook, 1979). She also traced series representing variation in the extent of colonial integration in respect of avicularia and vibracula, in terms of morphology, siting and its regularity; and in respect of ovicells in relation to the number of autozooids involved.

Cook (1979) concluded that "integrated, colony-wide functions may be much more common in bryozoan colonies than hitherto expected", and that an understanding of colony morphology was a prerequisite to revealing them. She pointed out, however, that there is no simple relationship between highly integrated morphology and colony-wide functions.

1.6.8 THE SPATIAL ARRANGEMENT OF ZOOIDS WITHIN MODULAR BRYOZOAN COLONIES

The actual spatial arrangement of all of the modules (zooids) within a bryozoan colony results from the astogenetic pattern of the species, invariably affected to some degree by various environmental factors. In the species considered in this thesis, in Chapters 5, 6 and 7, polymorphism is very well developed and polymorphic heterozooids may outnumber autozooids by a factor of 10. It is however the spatial arrangement of the autozooids which is fundamental in determining colony structure and morphology. The spatial disposition within a colony of the assemblage of

polymorphic heterozooids, however, may be of value in determining the way such colonies are structurally and functionally organised.

CHAPTER 2 – PRELIMINARY POPULATION STUDY OF *SCRUPOCELLARIA REPTANS*

2.1 INTRODUCTION

A literature search on *Scrupocellaria reptans* revealed little in regard to the general biology, or ecology, of the species. Lutaud (1953) looked in detail at the ancestrula, the development of young colonies, the lengths of internodes and bifurcations, and described the consistent branching pattern. I am aware of no study of the species which investigated colonies beyond the early stages of growth. Species descriptions within various regional bryozoan faunas give generalised accounts of colony form and the polymorph types present. Silén (1980) looked intensively at the relationship between the ancestrula and the substratum. Eggleston (1963) in the Isle of Man, found embryos in all months except April and October, with the greatest abundance between June and August. Embryos were present throughout the year but most abundant from June to October (possibly North Wales, Ryland, pers. com.), (Hayward and Ryland, 1998). I have found no reference to the settlement period, the rate of colony growth, mean or maximum colony size, or longevity.

2.1.1 Objectives

It seemed desirable, therefore, before looking in detail at the spatial arrangement of zooids within colonies and their structural organisation, to obtain some idea of the basic biological characteristics listed above. It seemed possible that some ecological information would also be obtained from such an investigation. It was intended as a very preliminary study, which aimed only to answer questions relating to the basic elements referred to above.

2.2 MATERIALS AND METHODS

A littoral population is present on the lower shore at Musselwick, in Milford Haven, Pembrokeshire (grid reference SM 819064), and this was sampled, generally monthly, at suitable low tides. Ideally, this sampling would have been quantitative.

However, the colonies of *S. reptans* exhibited a very pronounced clumped large-scale distribution. As a result, although suitable habitat – here largely *Chondrus crispus* – was present over much of the lower shore, *S. reptans* was found only in discrete areas of it. Any sampling using random quadrats would have led to very variable results, and sampling was therefore carried out simply by visual searching. This essentially revealed the larger colonies, and then all suitable substrate in that vicinity was collected. I thus tried to ensure that the sample was not biased in favour of the larger colonies. Only colonies still attached to their algal substrate, and which itself was still attached, were collected. The material collected was then scrutinised very closely in the laboratory, to ensure that all colonies were investigated. The aim of the study was to obtain a representative picture of the relative numbers of the various size colonies present at each sampling visit. It is apparent from Tables 2.3, 2.4 and 2.5 that small, often very small, colonies were collected by this method.

The 'Sea Empress' oil spill, 18/03/96, and its subsequent clean up, led to three abortive sampling visits; with the entire shore being covered in filamentous algae, and I feared that the population might have been wiped out. However, when the site was visited on 31/07/96 most of the filamentous algae had gone and the population was clearly still there. I had six months data before the oil spill, but an absence of any for the next four months. I therefore began a year long study, on 31/07/96, and as a result have two sets of results, one for the six months prior to the disaster and one covering a year beginning when the population was rediscovered.

Colony size was determined by counting the number of autozooids, so the results show the net result of growth minus any reduction caused by physical damage and/or predation. It was not possible to distinguish colonies that had been reduced in one way or another from those that had not. It subsequently became apparent that such reduction was common and extensive for a certain period of the year, and was an important element in any description of colony size changes over time.

The raw data detailing the sizes of colonies collected at each sampling date are in Appendix 'A'.

A sub-sample of the material obtained at each visit was used for the population study. Again it would have been preferable if this could have been quantitative, a certain number of colonies perhaps. The great range of colony sizes did not encourage such an approach and, to maintain the randomness of the sample, the material used was simply the complete contents of a number of collection containers.

The sizes of the colonies within these sub-samples were obtained by counting the numbers of autozooids in each colony. I had aimed to use at least 100 colonies from each sample, but this was not always possible when colonies were very large.

This data were used in two ways:-

- Firstly, the mean number of autozooids within a colony was calculated for each sampling date, and these were plotted as bar charts over time. It was apparent from the raw data that, for most of the year, a great range of colony sizes existed at any one time. This also needed to be quantified.
- Secondly, therefore, it was decided to group colony sizes, in terms of number of autozooids, into a limited number of size classes, which would provide a more detailed picture of the size structure of the population at each sampling date. This posed the problem of how to determine the number and ranges of these.

How useful are size classes here? Two completely separate factors are in operation, the importance of each of which varies temporally as does the relationship of one to the other.

- Firstly, for dichotomously branching species, the potential number of growing points doubles with each successive generation of bifurcations. This, while occurring in the early generations of internodes, cannot continue indefinitely, and the rate of increase then decreases at an unknown rate. It is, therefore, not possible for this aspect, to calculate size classes which will be appropriate for all colonies, at all stages of their growth.

- Secondly, in a temperate climate, a colony's rate of growth will vary with seasonal environmental parameters, level of available food, temperature, and perhaps salinity. Further, changes in colony size were not solely the result of how fast they were growing but also size reduction, caused by abiotic damage or predation, resulting in partial mortality. (This is a problem in respect of all modular colonies; Hughes and Jackson (1980) discussed this in respect of scleractinian corals, where the situation is further complicated by fission and fusion, elements absent here). Partial mortality must occur to some degree all the time, but the fact that for a certain period mean colony size fell considerably, clearly indicates the extent of its impact and that it varied greatly in extent over time.

These two elements are essentially independent of one another.

Despite these difficulties, it was necessary to try to put some structure into the range of colony sizes observed. Given that colonies initially grow exponentially, but then slow by a variable and unknown amount, it seemed that colony size classes used should attempt to reflect this.

When data had been collected it became apparent that although settlement occurred over a three-month period, for most of the year there was a great range of colony sizes at any one time. A very small minority of colonies grew very large but many only achieved a much smaller size before they reproduced and died. This also strongly militated against meaningful size classes.

Size classes were calculated on the premise that initially growth would be exponential (Ryland, 1976b) and that this growth rate would slow as colonies grew and competition for space reduced the rate of increase of growing points. The size classes were also initially calculated to produce nine such classes. The data were plotted as bar charts showing percentage occurrence of each class against time. It enabled the changes of colony size over time to be explored more closely: e.g. did such changes occur in certain size classes only, or across them all?

Attempts were made to calculate appropriate size classes in relation to an arbitrarily defined 'size class 1' (which was deliberately made very small, to facilitate the separation of newly established colonies). These size classes were very rough estimates of meaningful size classes. When the detailed study of one large colony of *S. reptans* was carried out (Chapter 5) additional data became available (although not directly translatable into size classes) which provided some guidance. It produced, admittedly for only one very large colony, data on the actual number of growing points and cumulative number of autozooids which existed in each generation of internodes. It thus provided information on a previously only estimated variable, changes in the number of growing points over time, although it did nothing to quantify changes in the rate of growth. Still ignoring variation in environmental conditions, size classes could be estimated on the assumption that a certain number of new generations of internodes would be produced since the previous sample was taken. Although such size classes would not incorporate any changes in growth rate over time which resulted from a change in environmental conditions, it was thought possible that some idea of this could be obtained from data obtained on successive samplings throughout the year. It became apparent, however, from this data that colonies frequently suffer partial mortality during the winter, and growth rates are then negative.

Size classes were calculated in relation to the number of autozooids present following each generation of bifurcations in the colony investigated in the detailed study described in Chapter 5. Given that that colony at its greatest extent had some 28 generations of internodes, nine size classes could be calculated on the basis of three generations of internodes equalling one size class, using the cumulative figures of the numbers of autozooids present.

The large colony investigated in Chapter 5 was collected in mid November and it is not unreasonable to assume from the data on settlement period that the colony would have originated in May to June, and that partial mortality would not have occurred to any great extent by this date. On these assumptions this colony had reached this size in less than six months.

2.3 RESULTS

(The raw data are in Appendix A).

2.3.1 Changes in mean and median colony size over time

Various parameters of colony size are shown in Table 2.1, below.

Sample Date	Day number	Number of colonies	Mean number of autozooids	Standard deviation	Median number of autozooids
09/10/95	75	123	690	1451	170
23/11/95	120	44	1169	2809	453
24/12/95	151	50	4107	5611	1696
22/01/96	180	102	2676	2822	1462
19/02/96	208	88	1746	1837	1229
19/03/96	237	134	1776	2228	1006
SEA EMPRESS OIL SPILL					
31/07/96	5	350	83	565	11
29/08/96	34	199	275	557	87
28/09/96	64	74	684	1267	268
26/10/96	92	75	1626	1853	978
12/12/96	139	52	2334	3303	1273
12/01/97	170	105	1366	2266	516
08/02/97	197	99	1159	1386	614
08/03/97	226	125	1972	3463	1150
09/04/97	258	87	2018	2034	1352
06/05/97	285	32	3200	3946	1552
22/06/97	331	237	187	667	15
23/07/97	362	1511	33	98	10

Table 2.1 Mean and median numbers of autozooids per colony at each sampling date

The most striking feature of Table 2.1 in respect of changes in mean/median colony size throughout the year is that they increased rapidly until the end of the year and then fell dramatically in January and February. They then increased to an even higher level (in the year-long study) in under a year, before crashing when colonies reproduced and died, in mid-summer. Table 2.1 also shows definite patterns of change in mean/median colony size over time and from these the following are apparent:-

- That this population of the species appears to be annual, with very low mean/median colony size only in late June and July.
- That it appears (on the basis of mean colony size) that there was not a simple increase in colony size throughout the year. Initially colonies grew very rapidly, but in the winter average colony size fell dramatically, and then increased again in the spring.
- That, for the six months period of the year included in both series of samples, the largest average colony size, reached in December, was much higher in the year before the oil spill. It is noticeable, however, that by mid March, the end of the six months period, there was very little difference between the two years. Indeed the average size of colonies at the beginning and end of the six-month period was very similar to those in the year long sample, and the pattern of change was very similar in the two periods.
- It is clear from the very large standard deviations that a great range of colony sizes was generally present at the majority of sampling dates.

The changes in mean colony size are shown graphically in Figures 2.1 and 2.2 below.

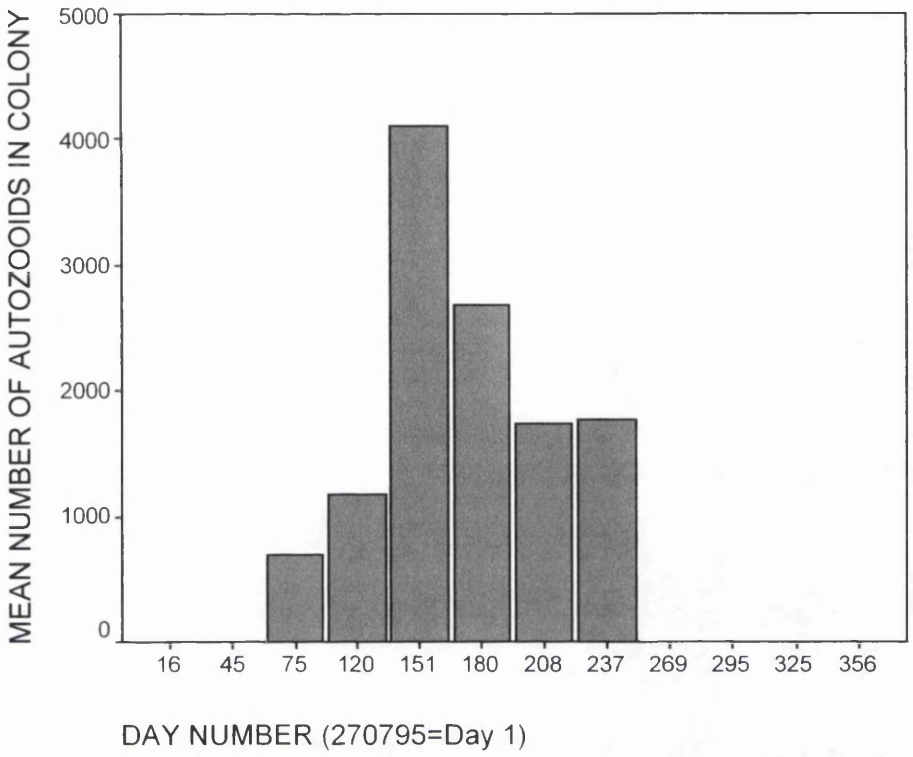


Figure 2.1 Mean colony size over the six month period commencing 09/10/95 and ending with the Sea Empress oil spill .

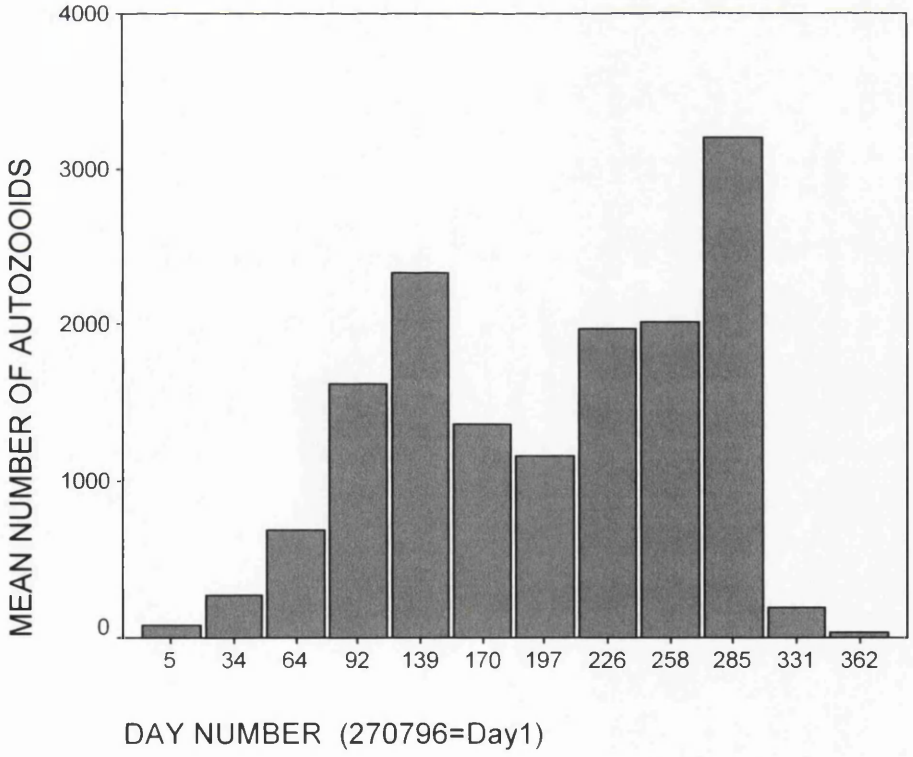


Figure 2.2 Mean colony size over the twelve month period commencing 31/07/96, four months after the Sea Empress oil spill.

Figures 2.1 and 2.2 above graphically illustrate the results discussed above. The species here is probably an annual, but growth occurs in two periods separated by three months when, unless there is an influx of new colonies mid-winter, growth appears to be negative. This is a very broad picture; and the breakdown of each sample into a number of size classes is necessary to clarify what is happening.

2.3.2 Temporal changes in size class structure

The figures below are calculated utilising the numbers of internodes and autozooids in the colony of the detailed study in Chapter 5 (see Materials and Methods, Section 2.2) and assuming that the number of new generations of internodes was constant over time. Whilst this is obviously not realistic in respect of changing environmental conditions, because the period covered excluded the mid winter, the more extreme variations probably did not occur within this period.

Sample Date	No. of col-onies	Size class								
		1	2	3	4	5	6	7	8	9
		Number of autozooids								
		1-30	31-340	341-2060	2061-4570	4571-8080	8081-13320	1332-17300	17301-18810	18811 +
091095	123	12	54	26	5	1	2	0	0	0
231195	44	4	39	48	7	0	0	0	2	0
241295	50	0	18	38	12	14	12	2	0	4
220196	102	1	13	45	18	16	7	0	0	0
190296	88	0	16	53	24	5	2	0	0	0
190396	134	2	21	48	18	9	1	1	0	0
SEA EMPRESS OIL SPILL										
310796	350	73	25	2	0	0	0	0	0	0
290896	199	27	54	16	3	0	0	0	0	0
280996	74	19	39	35	5	0	2	0	0	0
261096	75	8	19	47	21	4	1	0	0	0
121296	52	0	29	36	21	8	2	4	0	0
120197	105	5	35	39	13	7	0	0	1	0
080297	99	7	36	36	19	2	0	0	0	0
080397	125	8	19	38	28	6	0	0	0	1
090497	87	0	17	45	28	9	1	0	0	0
060597	32	3	12	41	16	19	6	0	3	0
220697	237	76	16	5	3	0	0	0	0	0
230797	1511	81	17	2	0	0	0	0	0	0

Table 2.2 Percentage occurrence of colonies of the different size classes, at each sampling date

It is clear from Table 2.2 that:-

- The very high incidence of very small colonies for only a two to three month period mid-summer, and their very low level of occurrence for the rest of the year confirms the species is, at least here, an annual.
- That since there is no influx of new colonies at the turn of the year which could account for the decrease in mean colony size at that time; there is a period of negative growth (partial mortality).

Table 2.2 shows that the changes in the percentage occurrence of the various size classes exhibited a less distinct pattern than might have been anticipated given the

single, if extended, period of settlement. This is in large part due to the substantial fall in mean colony size around the turn of the year. It is also true that the numbers involved for some sampling dates were perhaps rather small to be split into nine size classes. Nevertheless patterns are apparent, especially for the year-long period, and are more clearly seen represented graphically:-

- Figure 2.3 shows changes in the percentages of the various size class frequencies over time.
- Figure 2.4 shows for size classes one, three and five, changes in their percentage level of occurrence over time.

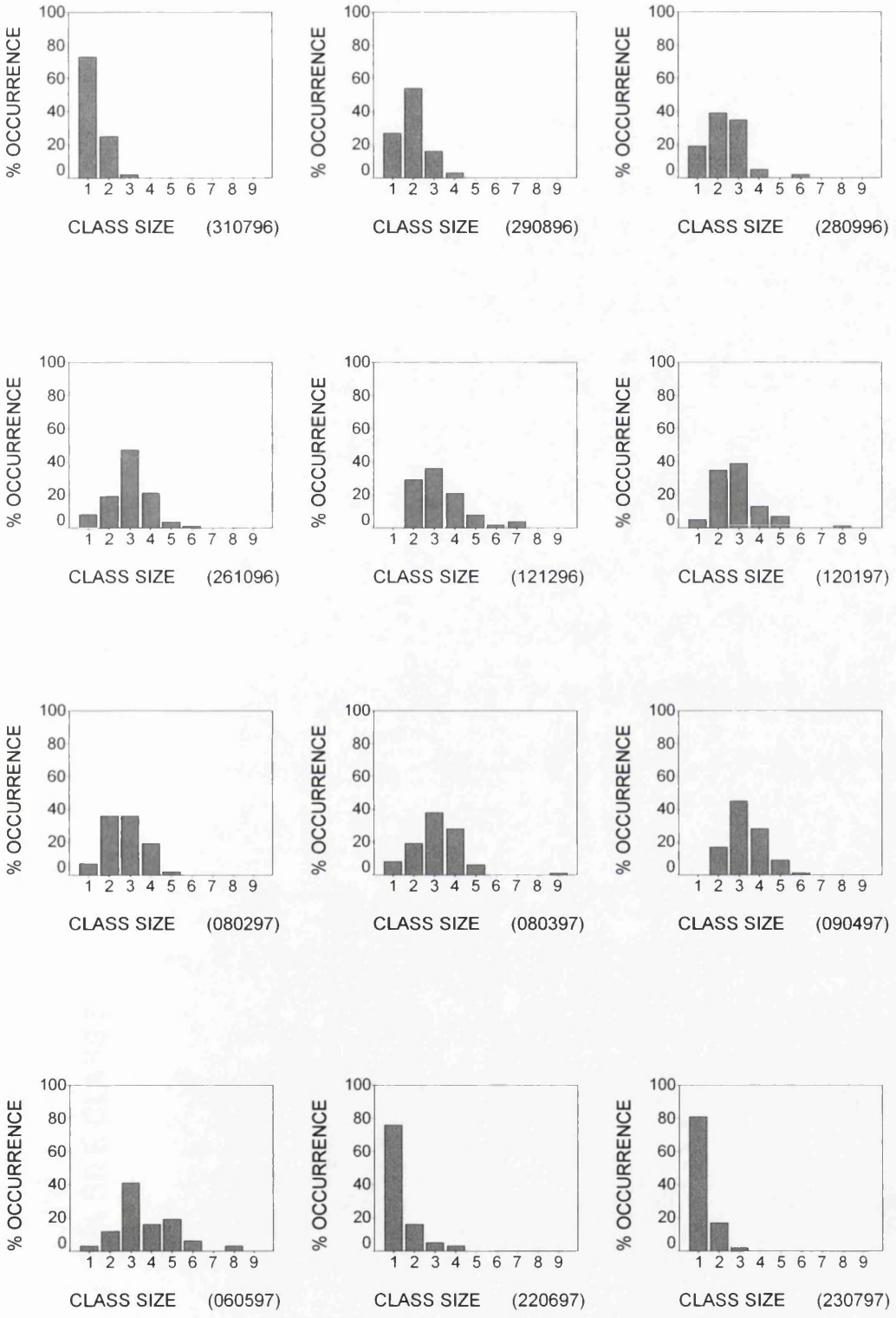


Figure 2.3 The frequency distribution of size classes, at each of the sampling dates over the year commencing 31/07/96

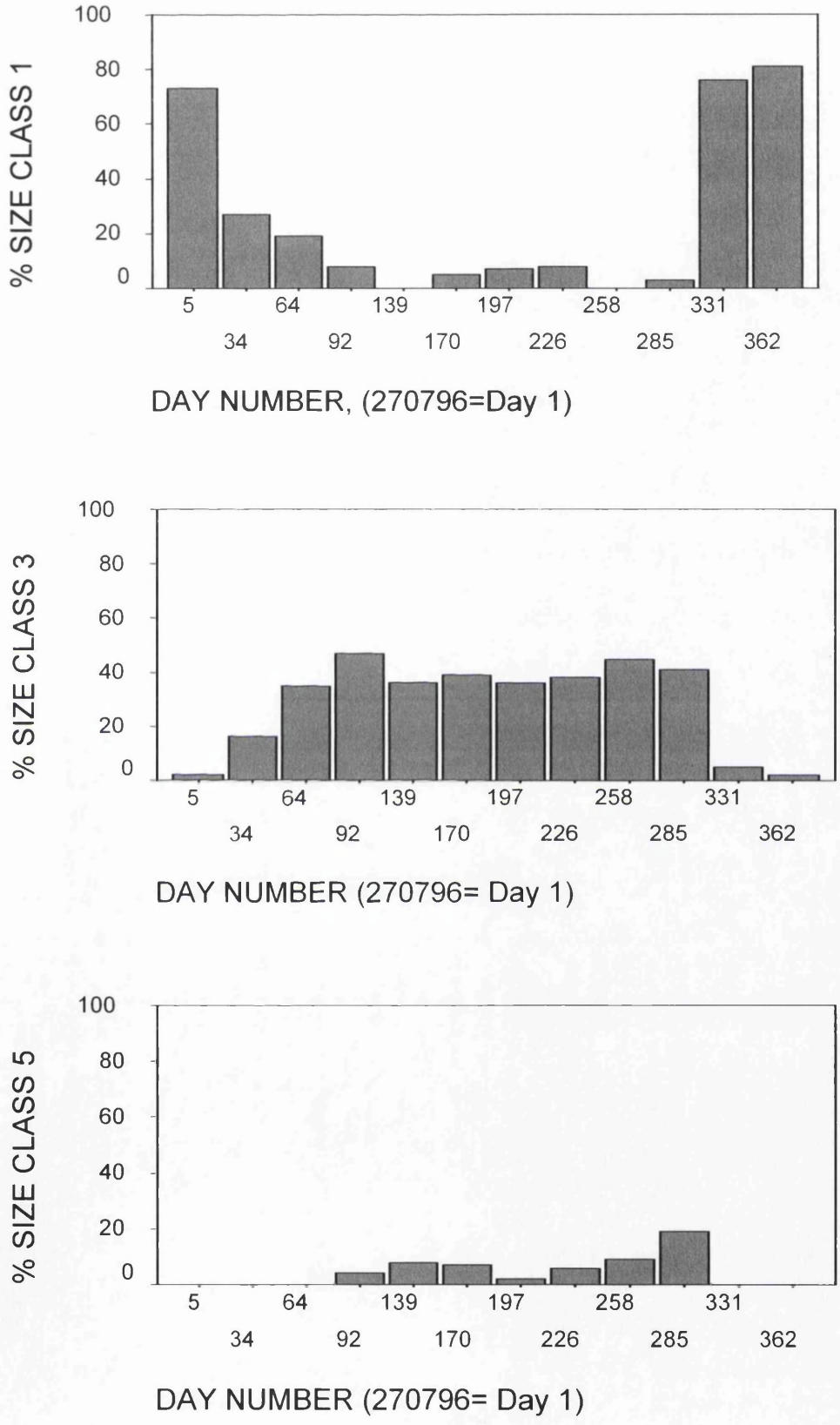


Figure 2.4 The percentage occurrence of size classes 1, 3, and 5 for each of the sampling dates over the year commencing 31/07/96

2.3.3 Range of colony sizes present at any one time

Although settlement was restricted to an essentially three to four month period; for much of the year the range of colony sizes to be found at any one time was considerable. Only for a short period in mid summer, when all colonies were small or very small was this not the case.

2.3.4 Final size of colonies

There was a great range of colony sizes prior to reproduction and only a very small fraction attained the largest or even the larger sizes. Probably 50% of colonies achieved < 2,000 autozooids, a few colonies attained more than 8,000, and a still smaller minority were of >20,000.

2.3.5 Settlement period

The percentage occurrence of the smallest colonies, very high only in samples taken in late June and late July indicate a limited settlement period. It was unfortunate that settlement commenced during a six-week interval between samplings between early May and late June.

2.4 OTHER OBSERVATIONS

2.4.1 Clumped distribution of colonies

As discussed under Materials and Methods, although there were extensive areas of *Chondrus crispus* (the preferred substratum here on the lower shore), *S. reptans* was found in abundance in certain areas and completely absent from others. The bryozoan exhibited a large-scale clumped distribution.

When small colonies were collected soon after settlement it became clear that clumped distribution also occurred on a small scale. Lengths of *C. crispus*, which had been separated from the parent plant as one or more colonies were present, for their autozooids to be counted, invariably proved to have more colonies present than was initially thought. Typically the vast majority of the frond was uncolonised but

where a very small colony was found, there often proved to be two, three or more colonies, with their first internodes very close together. There was no obvious micro-environmental characteristic related to these clusters.

2.4.2 Substrata

The vast majority of all of the colonies collected at Musselwick were on *C. crispus,* which existed in quantity low on the shore. Colonies were very occasionally found on other algae, such as *Mastocarpus stellatus* and *Ulva lactuca.*

2.4.3 Relationship to degree of water movement

The shore at Musselwick is Grade 5, 'fairly sheltered', on Ballantine's (1961) 'Biologically defined exposure scale', which graded shores around Pembrokeshire, from '1', 'extremely exposed', to '8', 'extremely sheltered'.

2.4.4 Colony habit

Colony habit on *C. crispus* was not really procumbent. The fronds of this alga are limited in extent, and colonies were not 'plume like' as some on filamentous red algae, but neither were they as procumbent as those on *Fucus serratus.* Colony habit reflects the extensiveness of their substrate available for rhizoid attachment.

2.5 DISCUSSION

2.5.1 Longevity

Very small colonies occurred at very high levels only in samples at the end of June and July, and for most of the year they occurred at a very low level. During the settlement period average colony size was very low (in the sample taken 23/07/97, of >1,500 colonies all were of < 1,000 autozooids) and large colonies were completely absent. This indicates that colonies do not survive for longer than one year. The littoral population at Musselwick, which therefore appears to be annual, was almost exclusively found on *C. crispus,* which is a perennial plant (Pybus, 1977).

2.5.2 Settlement period

Very small colonies were abundant in late July, but the much smaller numbers of such colonies found in late August and late September could be due to late settlement or slowly growing or damaged colonies. Settlement appears to be centred on June, July and the first half of August. Given the frequency and extent of partial mortality, and probable variation between colonies in their rate of growth, I doubt that the few very small colonies found throughout the rest of the year were the result of new settlement.

2.5.3 Temporal changes in mean colony size

It has been said that the number of autozooids in a growing bryozoan colony, in the absence of any environmental constraints, increases logarithmically during its growing season (Ryland, 1976b). Such constraints of course may take many forms: e.g. lack of space (Hayward, 1973) or seasonal factors such as falling temperature or low level of available food. Bushnell (1966) found that the arborescent form *Bugula turrita* exhibited geometric growth. Exponential growth, if it continues long enough, inevitably results in an environmental constraint, lack of space. Growth in *Alcyonidium hirsutum* (Hayward and Harvey, 1974; Hayward and Ryland, 1975; Owrid, 1988) exhibited geometric growth initially but then declined, resulting in the characteristic sigmoid curve.

For this population of *S. reptans* mean colony size increased rapidly until the end of the year, then fell dramatically for several months before increasing again in the spring and early summer to a higher level. It is known that old fronds of *C. crispus* degenerate and are thrown off in the winter (Rosenvinge, 1931) but whilst this may well account for a mid winter reduction in the population of *S. reptans,* it is unlikely to result in a reduction in mean colony size. This would require that larger colonies are disproportionally affected, which they may well be, given that the larger the colony the more likely it is to be damaged by water movement. This will be discussed briefly below in Section 2.5.5.

The evidence of the detailed study of a large colony of *Scrupocellaria reptans* (Chapter 5) indicated that for that colony of arborescent form, exponential growth, in terms of the number of points of growth, occurred for only a very short period. Growth then continued at an ever-declining rate. From the detailed study it appears that it may well be that the growth form of the colony is such that actual interference between 'aggregations' of internodes (lateral sections of a colony) may not occur. Limitations of space do however determine the lateral extent of the essentially small number of laterally discrete areas of extensive vertical growth which develop. This is discussed further in Chapter 5.

2.5.4 Range of colony sizes present at any one time

Although settlement did occur over a period of approximately three months, the range of colony sizes present at any one time was, for much of the year, far greater than might have been expected. This may have resulted from variations in growth rate and/or variations in the extent of damage and predation. Given the considerable decline in mean colony size in January and early February, and the fact that there was no influx of new colonies to account for this, partial mortality must have been a substantial factor. Do the variations in the percentages of the various size classes throw any light on this?

2.5.5 Temporal changes in size class structure

There were temporal changes apparent in size class structure but patterns were far less distinct than one might expect with a single, if protracted, period of settlement. Although the numbers of the larger colonies were always small there is evidence (see Table 2.2, and Figures 2.5 and 2.6) that to some degree it was these which were more severely affected, in both years, when whatever impacted on mean colony size in mid winter, occurred. It is of course true that the larger the colony, and therefore the greater its surface area, the more liable it is to suffer fragmentation (Hughes, 1989).

2.5.6 Final size of colonies

Although the final size of probably 50% of colonies consisted of < 2.000 autozooids, a few colonies consisted of >8,000 autozooids and a very small minority consisted of >20,000. I did consider that perhaps the very small number of very large colonies found could have been the result of two colonies growing together. In the colony of the detailed study (Chapter 5), however, for which a single internode of origin was not in doubt, the number of autozooids in the probably smaller 'half' of the colony which were counted, was ~9,500. The colony would therefore, have consisted of at least 19,000 autozooids.

The final size of colonies therefore varied enormously, for whatever reasons, and the largest size was seldom approached and very rarely achieved.

2.5.7 Rate of growth

No direct information was obtained on the rate of growth and the great variation in colony size could result from variation in rate of growth and/or damage or predation. For those colonies which did achieve large size this was often achieved in six months. On average half of this was lost in the winter and colonies tended to regain their former size after ~11 months. Growth rates for successful colonies were therefore very high from mid summer to the New Year, and only somewhat lower in spring and early summer. The relative importance of variation in growth rate, which may be genetic or result from environmental factors, and partial mortality, remain unknown, apart from the clear role of the latter in the substantial mid winter decrease in average colony size.

2.5.8 Clumped distribution

Aggregation of bryozoan colonies on both a large and small scale is well known. For the former, this may well be due to large-scale variation in certain environmental conditions. Whilst these may be less apparent to a researcher than to bryozoans, the lower shore at Musselwick appeared homogeneous. Where this is the case the

clumped distribution probably results from a combination of very limited larval dispersal and/or a tendency to aggregated settlement.

Clumped distribution on a small scale, aggregated settlement, is often related to microenvironmental variation, *Celleporella hyalina* (as *Hippothoa* sp.) in the depressions of *Laminaria saccharina* (Ryland, 1959), *Alcyonidium hirsutum* alongside the midrib of *Fucus serratus* (Hayward, 1973) and several species in association with the channelled side of *Pelvetia canaliculata* (Ryland, 1959). There are no such obvious vagaries in the surface of a frond of *C. crispus,* but settlement was very definitely clumped on a very small scale. Given that the clumping of young colonies was so very tight it is difficult not to believe that it results from aggregated settlement of larvae.

The distribution of many sessile marine invertebrates is heterogeneous on a small scale, and this is very often produced when larvae settle from the plankton; some settle in relative isolation, whilst others settle near conspecific individuals (Keough, 1983). Gregarious, or perhaps more accurately 'clumped' settlement, in *Bugula neritina* and *Celleporella hyalina* was demonstrated by Ryland (1976a). As cited above, the clumping of the latter species is probably related to environmental heterogeneity, and not true gregariousness. Keough investigated the settlement of *Bugula neritina* larvae, the colonies of which, at the southern Californian site investigated generally occurred in clumps of two to eight colonies. His study revealed that larvae reacted differently to sibling larvae than to those unrelated to them, which suggests a kin-recognition mechanism. Such aggregations of colonies afforded some protection from fish predators, at least for the individuals within the cluster, and suggest that the aggregation behaviour is best explained by a kin-selection hypothesis.

Given that very small colonies were often found very close together it is possible that large colonies were not always single colonies. Conversely, if they were, it follows that one colony in each original cluster survived at the expense of the others.

2.5.9 Substrata

The vast majority of all colonies collected at Musselwick were on *Chondrus crispus* which existed in quantity low on the shore. Colonies were occasionally found on other algae, notably *Mastocarpus stellatus* and *Ulva lactuca*, but although *Fucus serratus* was present in abundance no colonies of *S. reptans* were found upon it. Colonies collected for me from another littoral site, at Salcombe in Devon, were invariably on *F. serratus*. I do not know if *C. crispus* was present at that site. Gautier (1962) found much evidence that for many species there was often a close association with a particular algal substrate. It is also known that different populations of a species frequently have different preferred substrates at different geographical locations (Ryland, 1976b). It is possible that there is a hierarchy of preferred substrata, and if this is the case the Musselwick population points to *C. crispus* being preferred to *F. serratus*. Interestingly although the importance of bacterial film for larval attachment is well known, *C. crispus* has been shown to possess considerable anti-bacterial properties (Hornsey and Hide, 1976).

Ryland (1959) suggested that substrate selection could, especially for intertidal epiphytes, perhaps facilitate larvae settling at the appropriate level on the shore. At Musselwick *F. serratus* occurs immediately above *C. crispus* on the shore. In the sheltered shores around Dale and the western end of Milford Haven *F. serratus* occurs in a narrow band low on the shore, the lower limit of which is constant relative to chart datum, regardless of the degree of exposure of the shore (Moyse and Nelson-Smith, 1963). It is easy to imagine with greater exposure *S. reptans* would be able to extend further up the shore, and onto *F. serratus*.

2.5.10 Relationship to degree of water movement

It is quite likely that the degree of water movement is not a factor of great ecological significance in the distribution of *S. reptans*. As discussed in the introduction, the species is often found on *Flustra foliacea*, which occurs sublittorally, generally on coarse bottoms, most frequently in areas subject to strong currents (Hayward and Ryland, 1998). Ryland and Nelson-Smith (1975) described a rapids system at Cashla Bay, Galway Bay, in which *S. reptans* was found on *Laminaria digitata* and *L.*

saccharina, the latter species characteristic of sheltered conditions. Kitching and Ebling (1967) in their study of Loch Ine rapids, found *S. reptans* on boulders within the rapids, but only on those which experienced lower levels of maximal currents, and lower levels of mean tidal flow. My sampling site on the lower shore at Musselwick, in Milford Haven, was classified by Ballantine (1961) as a 'fairly sheltered' shore, grade 5, in a scale from 1 to 8.

2.5.11 Colony habit

Colony habit is generally described as 'creeping' (Hincks, 1880; Hayward and Ryland, 1998). Colonies which I have seen on *F. serratus* certainly should be so described. Colonies on the less extensive fronds of *C. crispus* are less so, and colonies on filamentous red algae, with a very restricted area for rhizoid attachment, still less so, exhibiting an almost 'plume-like' habit. Colony habit is strongly related to the extensiveness of the substratum available for rhizoid attachment.

Many of the larger colonies from Musselwick had a somewhat 'spiralled' appearance, as if a force had been applied from above, and then rotated through a short arc. I cannot envisage how or why this occurs, or whether this happens to the colony, or is achieved by it.

2.6 SUMMARY

Colonies of *Scrupocellaria reptans* at Musselwick, Milford Haven, were found on the lower shore, almost exclusively on *Chondrus crispus,* although other algae, notably *Fucus serratus,* were also present in abundance. Colonies exhibited a noticeably clumped distribution on both a macro and micro scale. On the former, they were often absent from considerable areas of their preferred substrate at this site. In respect of the latter, very young colonies were frequently found very closely aggregated, whilst the vast majority of the frond was uncolonised.

Although settlement of larvae and the initiation of new colonies occurred between mid May and early September, a great range of colony sizes was present through much of the year. This presumably results from differential rates of growth and damage. Only a very small minority of colonies achieved a very large size. The

species here is an annual, but mean colony size was almost as high after six months as it was after a year. In mid winter average colony size fell abruptly and considerably, and recovered in the spring, to above its former level. Large colonies were increasingly rare as the settlement period proceeded, and were totally absent by August, presumably having reproduced and died.

CHAPTER 3 - A SPECIES OF CELLULARINE BRYOZOAN NEW TO BRITISH AND ATLANTIC WATERS

(This Chapter, in somewhat different form, constituted the taxonomic element of:- 'The distribution, origins and taxonomy of *Tricellaria inopinata* d'Hondt and Occhipinti Ambrogi, 1985, an invasive bryozoan new to the Atlantic'; Dyrynda et al., Journal of Natural History, 2000, 34, pp.1993-2006).

3.1 INTRODUCTION

Colonies of a cellularine bryozoan were first found during August 1998, in and around Poole Harbour, Dorset. Material was initially collected on a detached frond of *Sargassum muticum*, and subsequently on attached plants and from inorganic substrata, littorally and in the shallow sublittoral. Colonies were numerous, often growing very closely together. They varied in size and in reproductive vigour, often being densely ovicellate. Although originally collected on the assumption that they were a species of *Scrupocellaria* (although such colonisation of *S. muticum* had not been previously observed), subsequent examination revealed them to be a species of *Tricellaria* not previously found in British or, it subsequently transpired, any Atlantic waters. A preliminary review of the modern literature revealed a remarkable degree of similarity between this material and some of the more recent descriptions of *T. occidentalis* (Trask, 1857), a species described previously from all around the rim of the Pacific but not outside it. D'Hondt and Occhipinti Ambrogi (1985) introduced *Tricellaria inopinata* for a species that had recently invaded the Venice lagoon. Gordon and Mawatari (1992) claimed that this new name was unnecessary as the range of variations which occurred within the material described by d'Hondt and Occhipinti Ambrogi fell within that known to occur in *T. occidentalis*, as described and figured by Mawatari (1951). Occhipinti Ambrogi and d'Hondt (1994) remained unconvinced and suggested a 'species complex' of *T. occidentalis, T. inopinata* and *T. porteri* (MacGillivray, 1889), a species first described from Australasia, which required further investigation.

The classification of the Candidae is largely based on the arrangement of the autozooids within an internode relative to each other and to the joints that separate internodes at bifurcations. Harmer (1923) distinguished a number of different types, which are useful taxonomically. The bifurcation of my material is as per the figure below.

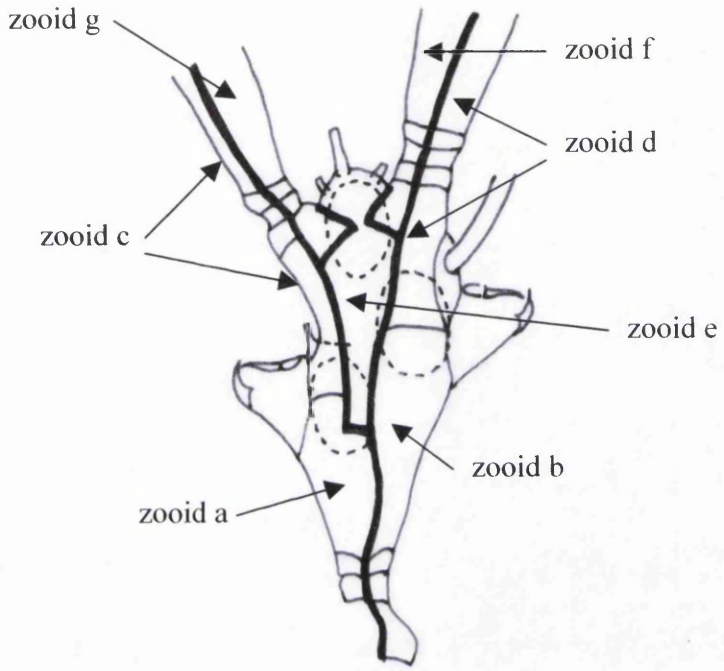


Figure 3.1 The arrangement of autozooids to each other, and to a bifurcation, in Harmer's bifurcation type 9, basal view (modified after Hayward and Ryland)

The presence or absence, number, morphology and spatial distribution of heterozooids polymorphs are also important in the classification of the group. (The lengths of internodes have also proved in this investigation to be of some value).

Harmer's work, which did much to clarify the classification of the family, was not published until 1923, and early descriptions of the species considered here were all as species of *Menipea,* as indeed were some of those that appeared after that date. In *Menipea* the arrangement of autozooids at bifurcations is of Type 17, 18 or 19 of Harmer's classification, and scuta are absent throughout the genus. In *Tricellaria* bifurcations are of Type 9, 10, 11 or 12, and scuta may be present or absent.

Few of the published descriptions refer to the bifurcation type as characterised in Harmer's classification. Mawatari (1951) ascribed his material to Type 5, although his figure suggests Type 9 or 10. D'Hondt and Occhipinti (1985) referred theirs to Type 9, as do I.

3.1.1 Objective

The objective of the study was to determine the identity of the material collected in Dorset. As the study progressed, other taxonomic problems became apparent which merited further investigation in regard to the two additional species, *T. occidentalis* and *T. porteri,* suggested to be within the species complex proposed by Occhipinti Ambrogi and d'Hondt (1994). I regard these as beyond the scope of this investigation and have confined myself, firstly, to noting material which requires reappraisal, as Occhipinti Ambrogi and d'Hondt's description was not reconcilable with mine; and secondly, to recording variations within any museum material which has been registered as conspecific.

3.2 MATERIALS AND METHODS

The comparative morphological study involved:-

- The collection of material by hand, littorally and sub-littorally, either complete with its algal substratum or removed from inorganic substrata. All of the material was preserved in 70% ethanol. Some of the material was partially cleaned, using fine brushes and some using diluted bleach, and examined using a binocular microscope.
- The examination of historical and recent literature descriptions and figures of the three species under consideration.
- The examination of museum paratype material of *T. occidentalis* and *T. porteri,* and recent museum material of *T. occidentalis* and *T. inopinata.*

3.3 RESULTS

3.3.1 Description of material

3.3.1.1 Colony form and composition

Colonies varied in size, the largest being ~15mm high x ~30mm in diameter, and in colour from white, through cream to buff, largely related to the age of the colony and the amount of material adhering to it. Internodes (branches) are often somewhat concave along their length, and the two produced at a bifurcation are angled relative to their precursor and each other. As a result the complete colony is a dense bushy mass, which is essentially circular in outline. The internodes of *Tricellaria* are unilaminar, essentially biserial (the two series of autozooids being staggered relative to one another), and have a single autozooid, centrally positioned, wedged between the distalmost pair (zooid e in Figure 3.1). All of the complete internodes so far examined contain an odd number of autozooids, from 3 to 19. Internodes branch dichotomously in a consistent manner but increasingly irregularly as the colony gets larger and variations in internode length increase. Each bifurcation is asymmetrical as a result of the way autozooids are arranged before it. The original ramus or 'stem' continues, but is deflected slightly to one side, and a new secondary ramus or 'branch' projects at a greater angle on the other side, slightly more proximally. Because internodes consist of an odd number of autozooids, each bifurcation is a mirror image of the one which precedes it.

3.3.1.2 Autozooids

Autozooids vary in size and shape in relation to their position within the internode, but all are elongate and taper proximally. The most distal autozooid of each internode is truncated proximally where it is wedged between the two autozooids proximal to it. The two most proximal autozooids of each internode are attenuated proximally where they span the joint with the preceding internode. Intermediate autozooids are, therefore, longer than the former and shorter than the latter. The

figures in Table 3.1, below, are based on measurements of 100 non-ovicellate and 100 ovicellate intermediate autozooids (20 of each from five different colonies).

	Non-ovicellate		Ovicellate	
	Mean	S.D.	Mean	S.D.
Autozooid length	473	54.5	398	27.9
Autozooid width	180	8.2	186	9.4
Opesia length	288	39.2	218	24.4
Opesia width	139	6.9	145	8.4

Table 3.1 Comparison of autozooid dimensions of non-ovicellate and ovicellate autozooids, in μm

Non-ovicellate autozooids are almost 20% longer than those with ovicells, and this difference is reflected in the different lengths of the opesia in the two.

The ancestrula is bath shaped, ~200 μm high x ~152 μm wide, lacks a scutum, and its opesia is virtually surrounded by ~8 thin straight spines. The autozooid budded from it arises disto-basally, very differently from the distal budding that occurs subsequently, and the connection between the two is much weaker than the common transverse wall that separates all other autozooids from their successor. I have found the ancestrula in situ only in very small colonies, a feature which is also characteristic of *Scrupocellaria reptans* and, I suspect, of many other cellularines.

3.3.1.3 Heterozooids

3.3.1.3.1 Spines

All autozooids have spines around the rim of the opesia. The limited amount of variation in their number and location relate to the position of the autozooid within an internode. For autozooids that occur biserially, the arrangement of spines in the two series are mirror images of one another. Generally there are three disto-lateral spines on the outer side of the autozooid. The most proximal is slender, often bifid, and angled somewhat over the opesia; the second is again slender, generally straight and more upright, and the third is generally more robust, longer, straight and more horizontal. On the inner side there are generally only two disto-lateral spines, both

slender, straight and upright. The axial autozooid, with a 'handing' the reverse of the autozooid proximal to it, has an additional long, thick, straight, near median spine, very prominent in the axil at bifurcations.

3.3.1.3.2 Scuta

All autozooids, except the ancestrula, have a scutum, a specialised spine, developed proximal to the midpoint of the inner lateral rim of the opesia. Initially it is a simple, straight, upright spine, but it subsequently bends laterally through ~90 degrees, overarches the opesia, and to a greater or lesser extent grows proximally, distally and laterally to form a shield of varying shape and extent. Whilst some species within the Candidae do not possess scuta, for those that do, scutal morphology is generally constant within a species and therefore useful taxonomically. In this species there is a considerable range of scutum size and morphology even within a single colony. Morphologies vary from a simple, slender or forked strut to an extensive cervicorn structure with a large-scale scallopped or spiky edge, via a number of intermediate forms.

3.3.1.3.3 Avicularia

Avicularia are numerous and varied within the Candidae, and they are found both frontally and laterally within the genus *Tricellaria.* In this species they occur only laterally, and are not present on all autozooids, their presence/absence correlating with several different features. Excluding the apical autozooid of each internode, on which they cannot occur, they were found, taking the colony as a whole, on probably 25% of them. They occur more frequently in the more proximal region of the colony and more constantly on the most distal pair of autozooids within each internode. They are sited disto-laterally and vary considerably in size, from 86 to 210 μm long, and from 57 to 172 μm wide, the largest forms occurring only on the most distal pair of autozooids of an internode. There is little morphological variation, although some are not attached throughout their length to the autozooid that gives rise to them. All are essentially triangular and largely directed laterally, although some are inclined partially frontally. The mandible and rostrum are hooked at their tips.

3.3.1.3.4 Rhizoids

Rhizoids are concentrated on the more proximal internodes. At bifurcations they occur proximal to the joints.

3.3.1.4 Ovicells

The sub-immersed hyperstomial ovicells which are positioned orthogonally to the branch axis are sub-globular and ~184 μm high x ~173 μm wide. The ectooecium has a number of pores, usually ~15, situated on a smaller number of radiating sutures. Because the ovicell in *Tricellaria* is produced by the autozooid distal to the one producing the embryo (Nielsen, 1985) the axial or apical autozooid of each internode is never ovicellate. The two autozooids proximal to it, have, distal to them, autozooids proximally traversed by the joints between internodes, and are rarely ovicellate. Whilst short internodes are generally infertile, the longest internodes are often completely ovicellate apart from the three autozooids referred to above. The embryos are deep pink in colour.

3.3.2 Descriptions of the three nominate species related to the examination of relevant historical and/or recent material

3.3.2.1 Introduction

How does the material described above relate to that described in the literature as *Tricellaria occidentalis*, *T. porteri* and *T. inopinata*, and how do these relate to one another? Most descriptions of these species are in accounts of the bryozoan fauna of a particular area and, as such, are often briefer than one would wish. They are also often frustratingly lacking in detail of the characteristic(s) crucial to identification, being written long before Harmer's (1923) delineation of the various bifurcation configurations. Dredged material, especially in the case of arborescent forms, often involves the collection of colony fragments rather than complete colonies, and this may well have adversely affected the completeness of resultant descriptions.

3.3.2.2 Historical and recent accounts within the literature, related where possible, to the examination of relevant historical and/or recent material

I shall describe what I believe to be three very different morphologies, and relate the most relevant descriptions to them. Other accounts will be considered chronologically once these three morphologies have been described.

Firstly, the morphology initially described by Trask (1857) as *Menipea occidentalis,* from the west coast of the USA, from Santa Barbara, California, to Cape Flattery, Washington. Whilst the description and figure are lacking in detail, Trask was very definite that the internodes of *M. occidentalis* consisted of three autozooids. Some idea of the form of the scuta can be deduced from the fact that he made no reference to scuta as such but only to stout, curved spines pointing upward and inward. (I have, unfortunately, been unable to locate type material of *M. occidentalis* and have been led to believe that it may no longer be retrievable from other conspecific material; D.F.Soule, personal communication). Hincks (1882) describing material from the Queen Charlotte Islands, British Columbia, and apparently unaware of Trask's description, erected *Menipea compacta* n. sp. form *triplex* on the basis of 'zooecia in triplets', although in 1884 he conceded that not all were such, some being of five or six. Paratype material (B.M.N.H. 99.1666 – Vancouver, Busk Collection) has been seen. I have also had access to other material of *T. occidentalis* from various localities on the west coast of North America (B.M.N.H. 77.3.7.5 – San Francisco, 1837; B.M.N.H. 1991.9.27.2.3. – Port Townsend, Washington State, 1991; B.M.N.H. 67.1.9.35 – Vancouver; B.M.N.H. 2.14.2 & 3 – NW. America, 1963). The majority of the material held by the B.M.N.H. as *T. occidentalis* (which is not all identical), is composed almost entirely of internodes of three autozooids, with a small number of fives toward branch tips. The autozooids are noticeably squat. A characteristic described by Hincks (1882; 1884) and also apparent in the B.M.N.H. material, is that the scuta are strut like or, if larger, still very moderate in size. Neither Trask (1857) nor Hincks (1882; 1884) described bifid spines and none were observed in the B.M.N.H. material. In that material two spines are prominent in the axil of bifurcations and lateral avicularia are numerous and large.

Secondly, the morphology first described by MacGillivray (1889) as *Menipea porteri,* from South Australia. Internodes were of five to seven autozooids, the most proximal of the three outer spines being clavate or bifid; scuta were extensive, somewhat reniform; and the lateral avicularia were rather scarce, often one per internode and sometimes even this was lacking. MacGillivray described and figured ovicells with a distal row of ectooecial pores, as opposed to scattered pores. I have examined paratype material of *T. porteri* from South Australia (including slides 65508 & 65509 – Rev. Porter collection; 65511 and 65513; Museum Victoria, Melbourne). Whilst I have seen ovicells in his material, which look from one side as if this is so, most ovicells appear to have pores scattered over their entire surface, although these are less apparent/numerous than in *T. inopinata.* (Whilst the majority of the paratype material which I have seen is as MacGillivray described it, two colonies are possibly of different morphologies).

[Interestingly in 1890 MacGillivray received another colony from Rev. Porter and in respect of this he noted that the scuta were wanting or small and clavate, and that the marginal spines were thicker and that none were bifurcate. Unfortunately he made no reference to internode lengths but his brief comments suggest that he was puzzled by these differences].

Thirdly, the morphology very fully described and figured by Mawatari (1951) as *T. occidentalis,* from the coasts of Japan, with internodes of three to nine autozooids, or more in distal internodes, one or two (?) bifid spines, and various scuta morphologies ranging from broad or forked struts (typical form) to large cervicorn forms with large-scale scallopped edges (var. *catalinensis*) via a number of intermediate forms. (He figured some 10 of these). Lateral avicularia were variable in size and numerous. Gordon (1986) and Gordon and Mawatari (1992) both described material from New Zealand, identical to the description of Mawatari, but with some bifid spines only in the proximal outer position. Finally, d'Hondt and Occhipinti Ambrogi (1985) introduced *T. inopinata* for a species that had recently invaded the Venice lagoon, distinguishing it from presumed *T. occidentalis* on the basis of avicularium size, ovicell morphology and proportions, and ecological differences. They made no reference to the characteristics that I believe separate *T.*

occidentalis and *T. inopinata* (see Table 3.2). Essentially their description is of material with this third morphology, long and varied internode lengths; some proximal external bifid spines; variable scuta morphologies ranging from strut-like to large cervicorn (but not reniform), via intermediate forms; and numerous lateral avicularia that vary in size. This is the morphology of my material.

I shall now consider chronologically the other cited accounts. The material described in these other accounts cited below can, where discrepancies are largely omissions rather than contradictions, often be straightforwardly assigned to one of the three morphospecies described above. However in some accounts the description and/or figures do not accord with the above scheme and a re-examination of the material is required.

Ortmann (1890) described material referred to *M. compacta* and also introduced var. *dilatata* for material which had kidney-shaped scuta, but which, in other respects, was identical to the nominate species. His figure, presumably of *M. compacta,* shows scuta, which are essentially 'T' shaped! This material needs reappraising.

Robertson (1905) describing material collected between San Diego and north to the Queen Charlotte Islands, described in addition to the nominate species *M. occidentalis,* a variety *M. occidentalis* var. *catalinensis*. The former was characterised as having internodes of three autozooids, with scuta being spine-like or slightly flabellate and, although spines were referred to, there was no reference to any of them being bifid. Var. *catalinensis* was introduced for colonies with internodes more often of five or seven autozooids, having large and fan-shaped scuta, with their edge divided and extended into a number of spiny processes, and with one or both of the spines that met over the upper part of the aperture sometimes being bifid. [This differs from a description of *T. inopinata* only in the reference to the occurrence on some autozooids of two bifid spines]. The bifid spines in her figure are all only one per autozooid and occur as the most proximal of the outer spines. This is as in *T. inopinata* and the fact that in this species some of the very variable scuta are simply forked struts seems a likely explanation for this. Robertson went on to say that the variations from the type species occurred rather constantly in specimens from the

south, but that she considered them insufficiently important to establish a new species. Robertsons's description and figures of the spines are somewhat confusing.

There is some ambiguity in Robertson's (1905) account as to the characteristics of var. *catalinensis* in that it can be interpreted as meaning that longer internodes, bifid spines and extensive scuta are present throughout the colony. It is only in the note at the end of the account, referred to above (that variations from the type occurred rather constantly in southern specimens) that there is a suggestion that variations of these features can occur within a single colony.

Yanagi and Okada (1918) describing Japanese material, stated that most of it conformed to *M. occidentalis* var. *catalinensis*. They clearly believed that *M. occidentalis* had only undivided scuta and var. *catalinensis* only divided ones, since they used this character to distinguish between the two in their key. They also noted the presence of some internodes of three autozooids in colonies of var. *catalinensis* and implied that this was at odds with Robertson's description.

O'Donoghue and O'Donoghue (1923) noted that their material, from Vancouver Island region, had some forms [colonies?] that approximated to *M. occidentalis*, but that a number of others were intermediate between that and the var. *catalinensis*. They noted, "none of them quite reaches the extremes exemplified in Robertson's sub-species".

Okada (1929) described material referred to *M. occidentalis* var. *catalinensis* from Mutsu Bay, Japan, and made reference to variations of internode length and to the variety of scuta morphologies within a single colony.

Silén (1941) described young colonies with the morphology ascribed above to *T. occidentalis,* but with some larger scuta, and older, larger colonies having, in their distal parts, the morphology ascribed to var. *catalinensis,* but with the scuta kidney-shaped and undivided! Because of this intra-colony variation he believed that the variety *catalinensis* was invalid. The reference to kidney-shaped scuta requires

further examination, especially in the context of variable scuta morphologies within a colony. (*T. porteri* has, I believe, all reniform scuta).

(It is worth noting here that proximal/distal variations in *T. inopinata* exist but are neither simple nor absolute. Whilst the longer, especially the longest, internodes are generally located distally, ovicells are generally similarly situated, and bifid spines and extensive scuta are more often found on ovicellate autozooids, internodes of five autozooids may be found very proximally, and bifid spines and extensive scuta are to be found on non-ovicellate autozooids within them).

Osburn (1950) describing material collected from Southern California north to British Columbia, still separated the nominate species, *T. occidentalis,* from var. *catalinensis*, with the former having internodes of three autozooids (occasionally five or seven) with scuta which were spur-like or a simple fork, and presumably simple spines (since there was no reference to any being bifid). In the latter, there is no reference to internode lengths, scuta varied from a curved spine to a broadly branched structure with a number of points, and bifid spines were present but not constant. He also referred to the intergrading of Robertson's characters within a single colony of var. *catalinensis.*

Bock (1982) described material referred to *T. porteri,* from South Australia, with internodes of three to nine autozooids. He neither described nor figured bifid spines, described elliptical scuta but figured ones much more irregularly shaped, and figured lateral avicularia more numerous than on the material of *T. porteri* that I have seen. This material needs reappraising.

Brock (1985), again from South Australia, figured *T. porteri,* and showed some bifid spines and a variety of scuta morphologies, including strut-like forms, and others larger with wavy or spiky edges, but none as being reniform. I believe that this material is *T. inopinata.*

Gordon (1986) described material from Nelson Harbour, South Island, New Zealand as *T. occidentalis.* The internodes are of three, five, seven or nine autozooids, the

most proximal outer spine is often forked, and scuta are very variable, awl-like, bifid, trifid or lobate. Gordon referred to the amount of variation within the species and that at least two varieties had been named, but that they seemed part of the intrinsic variation possible in the species. That is a description of *T. inopinata.*

Gordon and Mawatari (1992) described identical material found in a number of ports and harbours in New Zealand, again as *T. occidentalis.* They referred to earlier descriptions and misidentifications but did not appear to appreciate the differences between *T. occidentalis* (as described by Trask and Hincks) and their material.

Soule, Soule and Chaney (1995) described and SEM'd material from the Santa Maria Basin and Western Santa Barbara Channel as *T. occidentalis.* The internodes of their material were of three autozooids, but sometimes up to five or six (?). The scuta varied from a simple spur, usually in northern specimens, to an expanded multi-pronged structure, usually in southern material. It is not clear if individual colonies had only one or the other or if there were colonies with a range including both. Spines were described as three on the outer and three on the inner margin, with no reference to any being bifid. Their SEMs show rather clavate, non-bifid spines, two of which are prominent in the axil at bifurcations, and slender scuta. These features and the squat nature of the autozooids are *T. occidentalis* like, and utterly different from *T. porteri* and *T. inopinata.* The median suture on the ovicell referred to in the text as sometimes being present, and clearly visible on one of the SEMs, is however something that I have not observed in any of the material I have seen of any of the three species.

3.4 DISCUSSION

Before considering the characteristics which distinguish the three species being considered and the sequence of events that resulted in so much taxonomic confusion, it is worthwhile to say something with regard to the pattern of branching which occurs in *T. inopinata* and indeed *T. occidentalis, T. porteri* and, I suspect, other cellularine Bryozoa. It was described by Lutaud (1953) in respect of *Scrupocellaria reptans.*

It has often been observed that in many biserial cellularines successive bifurcations are mirror images of one another. This cannot occur without the 'handing' of the arrangement of autozooids immediately proximal to the bifurcation being reversed at each successive bifurcation. Given the arrangement of autozooids within an internode, a number of staggered pairs and a centrally placed apical autozooid, only internodes consisting of an odd number of autozooids can bring this about. References within the literature to internodes of an even number of autozooids are problematical, and almost certainly wrong, none have been seen in my material or in any of the museum material that I have seen of the other two species.

The characteristic branching pattern and the fact that all internodes are morphologically identical (apart from length and 'handing') was not apparently appreciated by a number of early workers. Robertson (1905), Yanagi and Okada (1918), and Okada (1929), refer to primary, secondary and tertiary branches, although neither how they differed nor how they came about was made clear. Mawatari (1951) also referred to the branches thus.

Related to the above, the fact that ovicellate and non-ovicellate autozooids differ considerably in length, and in the consistency of the relationship of the autozooids immediately proximal to the bifurcation, it is not surprising that ovicellate autozooids generally occur in staggered pairs.

Cheilostomate taxonomy is heavily reliant on the determination of morphospecies using qualitative and/or quantitative exoskeletal characteristics. Jackson and Cheetham (1990) using breeding experiments and protein electrophoresis, investigated three distantly related cheilostomate genera in respect of the reliability of morphospecies. They concluded, "morphospecific identity of cheilostomes is heritable and that morphospecies are genetically distinct with no indication of morphologically cryptic species". Morphologic species appeared to be good biologic species. They noted that cryptic species were a common occurrence in soft-bodied Ctenostomatida (Thorpe et al. 1978; Thorpe and Ryland, 1979).

Lidgard and Buckley (1992) investigated phenotypic variation and the likelihood of morphologically cryptic species in Recent populations of *Adeonellopsis yarraenensis* from Australia and New Zealand. Using principal component analysis, cluster analysis and discriminate analysis, they concluded that there were actually probably five separate species. They went on, "these results also suggest that an alarming number of cryptic species may exist within currently accepted yet poorly defined species boundaries, particularly among taxa that are morphologically variable and geographically widespread."

The reliability of morphospecies in determining actual biologic species would appear to be very variable taxonomically, and perhaps also in relation to precision of the identification of the morphospecies. Nevertheless many morphospecies are readily identifiable according to the presence of highly consistent features (Hayward and Ryland, 1998). *T. inopinata*, on the other hand, is characterised in certain respects by a high level of morphological variability within an individual colony. This appears to have been the source of considerable confusion within the Pacific literature pertaining to *T. occidentalis* (Trask), *T. occidentalis* var. *catalinensis* (Robertson), and to a lesser extent *T. porteri* (MacGillivray).

In essence I believe that much of the confusion in respect of the identities of the material described in the accounts cited above has its origins in Robertson's erection and description of *T. occidentalis* var. *catalinensis.* Firstly, I believe she was in error in her expressed intention to differentiate a variety from its nominate species, rather than to distinguish between two distinct species. Within the literature cited, no material resembling the material originally described by Trask, as *M. occidentalis*, or Hincks, as *M. compacta* form *triplex,* has been described from New Zealand, Venice or England, which supports the view that var. *catalinensis* is not merely a variety but a separate species, *T. inopinata.* Secondly, the ambiguity of that description led to subsequent workers noting the occurrence of *T. occidentalis'* characters within colonies of var. *catalinensis*. Yanagi and Okada (1918) distinguished *T. occidentalis* from var. *catalinensis* on the basis of scuta morphology, and were concerned that internodes of three autozooids were to be found in colonies of var. *catalinensis.* O'Donoghue and O'Donoghue (1923) felt that "none of them [the colonies] quite

reaches the extremes exemplified in Robertson's sub-species". Okada (1929) again noted the variations in internode lengths and scuta morphologies within a single colony.

This led Silén (1941) to declare that if a single colony of *T. occidentalis* var. *catalinensis* exhibited all of the characteristics of both the nominate species and the variety, then that variety was invalid. As a result, firstly the distinction between *T. occidentalis* and *T. occidentalis* var. *catalinensis* was lost, and subsequently material very different from that originally described by Trask was assigned to the nominate species. This, I suspect, led Mawatari (1951) – although referring in his description to the simple scuta (typical form) and broad flabellate forms (var. *catalinensis*) – to ascribe his material to *T. occidentalis*, with Gordon (1986) and Gordon and Mawatari (1992) doing likewise.

What I believe was not appreciated was that *T. occidentalis* possessed a more limited range of certain characteristics, internode lengths, spine and scuta morphologies, than did var. *catalinensis.* The number of prominent spines in the axils of bifurcations, two in *T. occidentalis* and one in *T. occidentalis* var. *catalinensis,* is, I suggest, the most constant single characteristic distinguishing the two.

Thus, three distinct species seem to have been confused in these previous accounts:-

- In the first, the internodes are almost exclusively of three autozooids; all scuta are simple and poorly developed; no spines are bifid, two are prominent in the axil of a bifurcation; and lateral avicularia are numerous: this is *T. occidentalis.*
- In the second, internode lengths are longer and more varied; some proximal outer spines are bifid; scuta are never strut-like, are always extensive but are reniform rather than wavy or spiky edged; and lateral avicularia are sparse: this is *T. porteri.*
- In the third, the internodes are very variable in length, including some which are very long; some proximal outer spines are bifid, only one is prominent in the axil of a bifurcation; scuta are very variable, ranging from a broad or forked strut to a large wavy edged cervicorn (but not reniform) structure, through a variety of intermediate forms; lateral avicularia occur on ~ 25 % of autozooids, excluding apical ones, and vary in size: this is *T. inopinata.*

Table 3.2, below, summarises the diagnostic features separating the three species).

	T. inopinata	*T. occidentalis*	*T. porteri*
Internode lengths-(number of autozooids)	Very variable-Observed range 3-19	Majority of 3, some of 5	Variable-observed range 3-13
Marginal spines-(excluding scuta)	Proximal external spine sometimes bifid	Proximal external spine never bifid	Proximal external spine sometimes bifid
Number of spines prominent in branch axils	1	2	?
Scuta	Very variable even within a colony-from a slender strut, perhaps forked, to an extensive cervicorn structure with a wavy/spiky edge	Slender or at most slightly spatulate	Consistently large and reniform
Lateral avicularia	Distribution complex, more common in proximal internodes and on distal autozooids within an internode. Variable in size	Present on the majority of non-apical autozooids	Present on a minority of non-apical auto-zooids

Table 3.2 Diagnostic characteristics distinguishing *Tricellaria inopinata, T. occidentalis* and *T. porteri*

The problematical accounts within the above and the fact that the B.M.N.H. material of *T. occidentalis* and the paratype material of MacGillivray's *T. porteri* both contain some colonies of probably different species, strongly suggest that the story is not yet complete. However, I believe that I have characterised *T. inopinata* and differentiated it from the other two species of the complex put forward by Occhipinti Ambrogi and d'Hondt (1985), *T. occidentalis* and *T. porteri.* Whether or not these two species, especially the former, includes one or more sibling species, remains to be determined.

CHAPTER 4 – MATERIALS AND METHODS

4.1 INTRODUCTION

For modular colonial organisms there are particular problems, which arise in any attempt to give a comprehensive description of the spatial arrangement of the constituent zooids within a colony. For bryozoans generally, colonies consist of a number, often a very great number, of zooids. Further, these will almost inevitably vary in form, zooidal polymorphism being characteristic of the vast majority of species, most notably and extensively in those having various erect morphologies. In all non-encrusting species, such as the arborescent *Scrupocellaria reptans* and *Tricellaria inopinata* considered in this study, the situation is considerably more complex than for encrusting species. A three-dimensional colonial morphology allows their constituent zooids to be spatially arranged in a number of different ways. It should be noted, however, that for erect bryozoans, in terms of their large-scale colonial morphology, only a limited number have been evolutionarily successful (McKinney and Jackson, 1989). These forms must have characteristics which have contributed considerably to the success of such species, but I suspect that other, less obvious, characteristics remain undescribed.

The original intention had been to concentrate attention on *Scrupocellaria reptans*, and then perform a similar study on a congeneric species, to establish if any newly discovered characteristics occurred more widely. The chance discovery of *Tricellaria inopinata,* in a closely related genus and a recent addition to the British fauna, caused me to rethink this. It became apparent during the investigation necessary for its description that, being closely related to *S. reptans*, it possessed a number of similar characteristics, but also that the two species were different in a number of respects. This invited a comparison. If previously undescribed characteristics emerged from the investigation into *S. reptans* should also be present in *T. inopinata,* this would be even stronger evidence of their more widespread significance. Conversely, if such characteristics differed in the two species, additional criteria in species differentiation would have been revealed.

4.1.1 Aspects of the spatial arrangement, within a colony, of autozooids and heterozooids, in erect cellularine Bryozoa

Underlying all scientific investigation is the belief that, however complicated it may be and how many disparate elements it may involve, there is an underlying order to the natural world. One manifestation of this, which is apparent in many colonial organisms such as Bryozoa, is the existence of pattern (Cook, 1985). The premise for this investigation is the belief that in many Bryozoa, especially erect forms, the spatial arrangement within a colony of all zooids, both autozooids and heterozooids, may exhibit characteristics which could be described with greater definition than is generally the case. This could reveal previously undetected aspects of colonial structure and organization, and perhaps suggest new lines of enquiry.

The spatial arrangement of autozooids is clearly fundamental to colony structure, and, by virtue of their monomorphic nature, was also always the more likely to lead to direct results. The spatial arrangement of polymorphic heterozooids is clearly secondary to that of the autozooids from which they develop. It is also, by virtue of the number of different forms, and the various factors involved in their occurrence, much more difficult to deduce, from their observed spatial distributions, why they are as they are.

I contemplated reviewing the literature in respect of the lengths of internodes which occurred in cellularine arborescent bryozoans. The number of autozooids within internodes of arborescent bryozoans is generally described as being within the range of 'X' to 'Y'. I have always assumed that these numbers referred to the number of autozooids in complete internodes, i.e. those which have bifurcated into two new internodes. I have only looked in depth at two such species. In one, *Tricellaria inopinata* (d'Hondt and Occhipinti Ambrogi, 1985), internodes were described as having between 6 and 10 autozooids. In the second, *Scrupocellaria reptans*, in the most recently published account (Hayward and Ryland, 1998) internodes were of between five and eight, but up to 12 autozooids. In both species, excluding the very first internode of a colony of *S. reptans,* which may be of four autozooids, I have failed to find a single complete internode of an even number of autozooids, and for reasons which will be detailed later, believe that they are unlikely to be found.

Further I have found internodes, of both species, of a lesser and greater number of autozooids, than the ranges quoted. These inaccuracies did little to encourage a review of the literature to obtain a broad survey of such information.

The presence or absence of various heterozooidal polymorphs is an integral part of bryozoan classification and is therefore invariably included in species descriptions. The vast majority of such descriptions are to be found in regional faunas, and it is clearly not feasible for each to be exhaustive. As a result, however, there are rarely attempts to quantify presence beyond 'rare', 'uncommon' or 'abundant'. References to patterns of distribution within a colony are uncommon, references to variations in morphology are more frequent, but there are few attempts to bring the two together. The two most notable exceptions to the above remarks, that I have seen, are Harmer's 'Cellularine and other Bryozoa' (1923) and Hastings' 'Discovery Report' (1943). Both refer extensively to the Candidae, although not very extensively to the two genera considered here.

I know of no precedent for an investigation of this type and it was, I believe, important to approach the search for possible patterns, trends or correlations, with an open mind. I recorded as many parameters as possible without any preconceived ideas as to their utility.

The precise spatial arrangement of all zooids, both autozooids and heterozooids within the colony, needed to be recorded if any patterns were to be discerned. For autozooids this involved their arrangement within internodes and the arrangement of internodes within the colony. For heterozooids, it involved their occurrence, morphology and distribution, both in relation to the position of their autozooid of origin within internodes, within the colony as a whole, and in relation to each other. It should be remembered that, in addition to the spatial aspect of zooidal occurrence, there is also a temporal aspect. Whilst autozooids can only develop in the proximal to distal sequence within each internode, the developmental sequence of polymorph production is not similarly constrained. Preliminary examination suggested that most of the adventitious polymorphs develop immediately their autozooid has been formed, but that rhizoids, although developing initially in this fashion, may

subsequently exhibit delayed development. It is, therefore, conceivable that other polymorphs could also exhibit delayed development.

4.1.2 Definitions of newly introduced terms

Before discussing the requirements of a coded recording scheme, which I believe is essential for such an investigation, it is necessary to define certain terms which I have introduced to define certain previously undescribed, or unused, characteristics.

Autozooid number

Autozooids are numbered from proximal to distal within the internode.

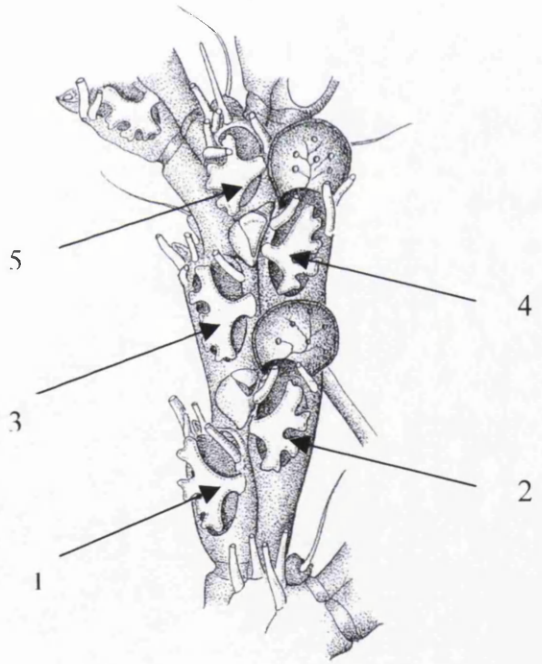


Figure 4.1 Autozooid numbers within an internode (modified after Hayward and Ryland)

Autozooid position.

Autozooids are designated 'apical' (the single terminal zooid in the internode); 'sub-apical' (the pair of zooids contiguous with but proximal to the apical zooid); and 'proximal' (all zooids proximal to the above three), as shown in the figure below.

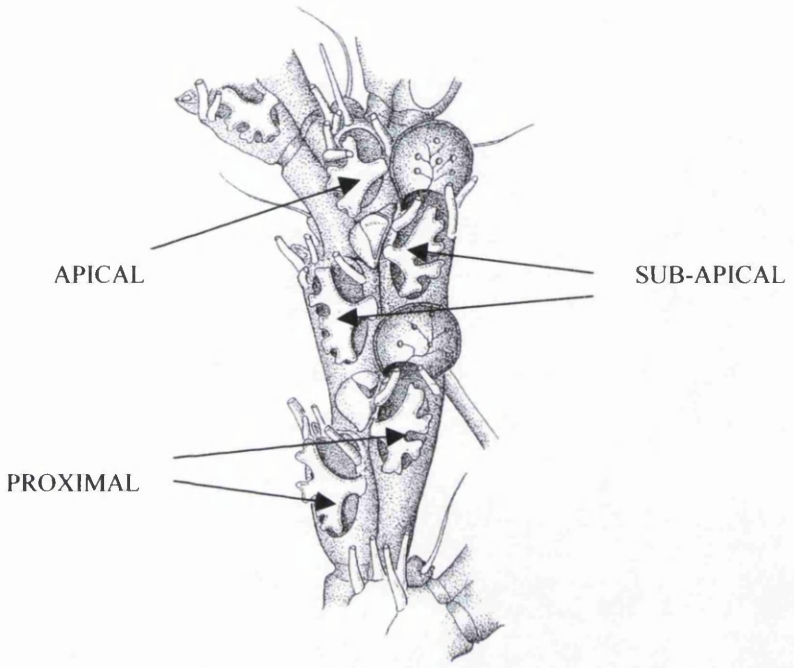


Figure 4.2 Autozooid positions within an internode (modified after Hayward and Ryland); working back from the apical autozooid

'Stem' and 'branch' internodes

At bifurcations 'stem' internodes develop more distally and deviate at a shallower angle than 'branch' internodes, as in the figure below.

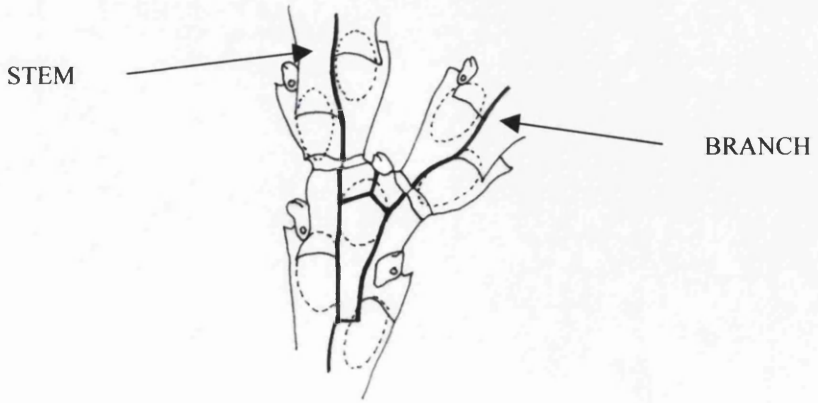


Figure 4.3 'Stem' and 'branch' internodes at a bifurcation (modified after Hayward and Ryland)

'Stem' and 'branch' internodes have generally been described as primary and secondary rami; cumbersome for repeated use. 'Branch' has been generally been used interchangeably with 'internode', and 'branches' in respect of a number of internodes. I am using the term in a very specific way.

Internode generation

The very first internode is a, indeed the, first generation internode. When it bifurcates, the two internodes it gives rise to are second generation, and so on, as per figure below.

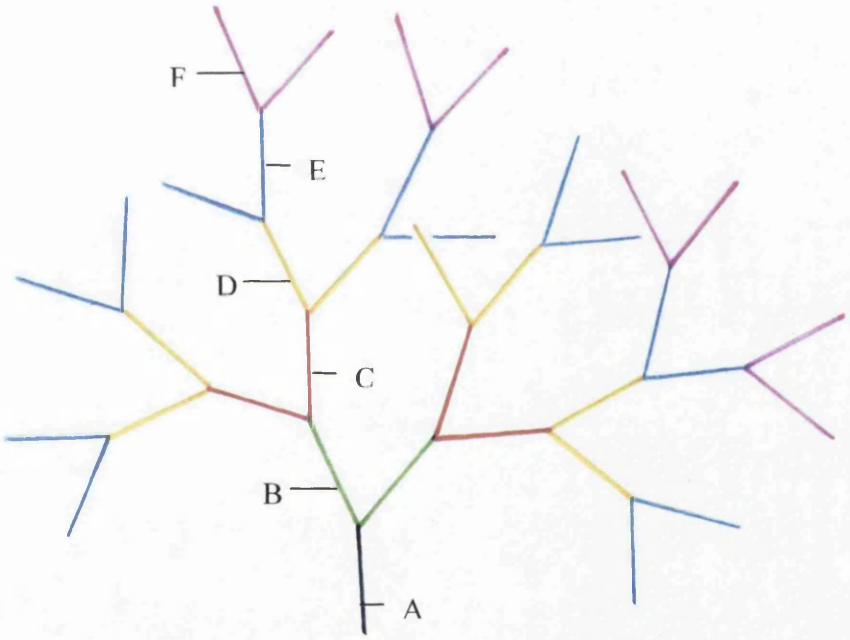
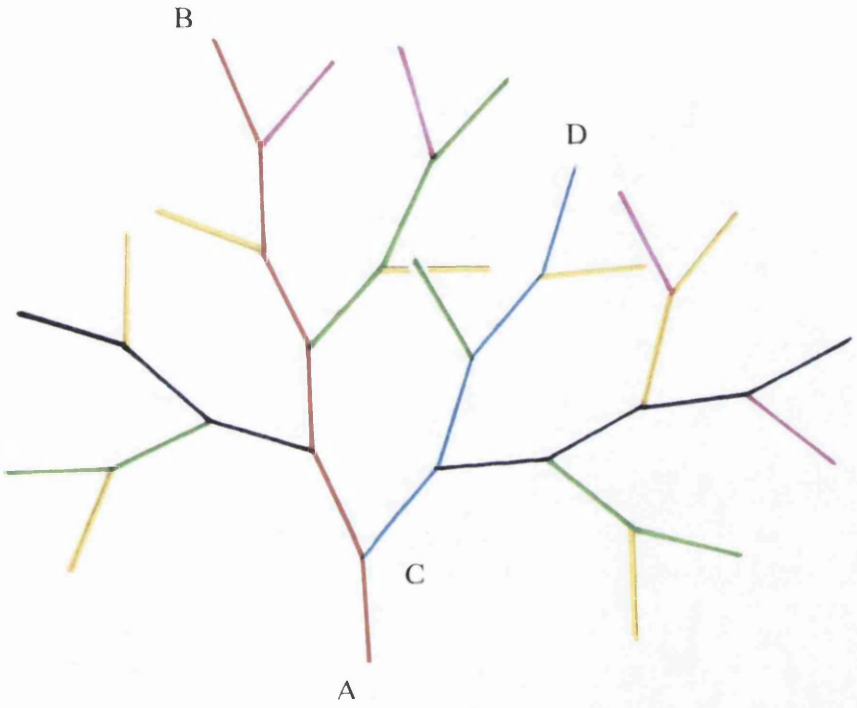


Figure 4.4 Internode generations

(It is of course true that, given the variations in internodes length, an internode generation, especially in the distal region of a colony, does not absolutely describe internodes that have developed simultaneously, but it gives some measure of vertical, and temporal distance, from the origin of the colony).

Stem sequences

'Stem sequences' are sequences of internodes each of which deviate from the direction of their predecessor by the smaller of the two possible angles. All, except the one originating from the very first internode, by definition begin with a 'branch' internode and continue with a series of 'stem' internodes, see figure below.



'A' -'B' main 'stem sequence', the very first internode and continuing with 'stem' internodes

'C'-'D' a 'stem sequence' originating with a 'branch' internode (in the second generation of internodes), and continuing with a series of 'stem' internodes

Figure 4.5 'Stem sequences'

'Stem sequence' is a concept which enabled a previously undescribed aspect of the arrangement of internodes within a colony to be described, and has proved very useful.

Aggregation(s)

'Aggregation', was used to denote the essentially discrete assemblage of internodes which consisted of a long 'stem sequence', together with all of the internodes (beyond the proximal region of a colony) which developed from it.

NB. I have in two respects used terms which have a generally accepted usage in respect of bryozoans, in a more specific way;-

- Firstly 'proximal' and 'distal' refer to the sides towards and away from the ancestrula respectively. Solely in respect of autozooids within an internode I have referred to all autozooids other than the apical and sub-apical pair as 'proximal'.
- Secondly 'branches' frequently refer simply to a number of internodes. I have used 'branch' specifically to differentiate such an internode from a 'stem' in relation to the angle of deviation at a bifurcation.

4.2 THE REQUIREMENTS OF A CODED RECORDING SCHEME

The use of a coded recording scheme enabling the relative spatial positions of all zooids within a colony to be recorded was made possible by two characteristics of both species. Firstly, internodes consist of autozooids with an identical front and back orientation; and secondly all branching is by simple bifurcation. As a result a colony can be described as if it were two dimensional, in terms of designating the precise location of each zooid within the colony.

Such a coding scheme needed to facilitate the recording and retrieval of information in as flexible way as possible. The aim was to arrange the data in such a way that correlations, associations, trends and comparisons could be sought via the computer quickly and easily, enabling a multiplicity of possibilities to be explored. In essence, it needed to allow the isolation of each individual factor, but also facilitate the investigation of synergistic effects.

Ideally designations would have included all of the information listed below, but in practice some compromises were necessary. Time was limited and a balance needed to be struck between approaching the investigation without preconceptions, and making the most effective use of the time available.

Autozooids required designations which identified:-

- Their numerical position within an internode.
- Their position relative to a bifurcation.
- Whether they were in the internal or external row within an internode.

Internodes required designations which:-

- Identified their 'generation' within the colony (from the ancestrula).
- Identified their lateral position within their 'generation'.
- Indicated the number of autozooids within them.
- Indicated whether they were complete or not.
- Indicated whether they were a 'stem' or 'branch'.
- Identified their distance from the growing edge.

'Stem sequences' required designations which identified:-

- The number of generations within them.
- The generation of their origin.
- The numerical position of each internode within its 'stem sequence'.

The usefulness of 'stem sequences' only became apparent after the preliminary study, and their details were added to the data sheets subsequent to the original data recording.

Polymorphs required designations which:-

- Identified precisely the autozooid that produced them.
- Indicated, if more than one, how many there were.
- Indicated, if variable in form, their morphology.
- Indicated, if variable in size, their size.

Ovicells required designations which identified:-

- The autozooid that they related to.
- The autozooid that produced them.
- Their position relative to the growing edge.

(Ovicell production also, perhaps, has a temporal aspect. Are they produced synchronously, or if not, in which direction does development proceed, distally or proximally, or perhaps in some other fashion?).

4.3 A CODED RECORDING SCHEME TO DESCRIBE, WITHIN A COLONY, THE SPATIAL ARRANGEMENT OF ITS CONSTITUENT AUTOZOOIDS AND HETEROZOOIDS

Colonies are unilaminar, with a frontal and a basal face with all lophophores emanating from the former. They are composed of internodes, which are biserial, with two rows of autozooids, staggered relative to one another, with a centrally placed autozooid wedged between the distalmost pair. The characteristic form of the colony, resembling a small bush, results from the bifurcations, which occur when an internode reaches maturity, and the angulation between internodes where this occurs. Each internode is separated from those that precede and follow it by a joint of chitinous tubes (node) and an uncalcified band.

All internodes can be considered identical in overall form, except that they exist in various lengths corresponding to the number of autozooids they contain, and are 'handed', according to whether the first autozooid is in the left or right hand series of autozooids. It is also possible to introduce another element, in respect of their relationship to the bifurcation that resulted in their formation. Bifurcations are asymmetrical in relation to the internode that precedes them. Of the two internodes produced, one is a slightly deflected continuation of its predecessor, the primary ramus, and the second, which deviates at a greater angle, the subsidiary ramus. For ease of use, as I refer to these frequently, I designate them, 'stem' and 'branch' respectively (see Section 4.1.2.3). Although as colonies grow they cease to be two dimensional, since all internodes have a frontal and basal surface with a consistent orientation, and all branching occurs as simple bifurcations, they can be identified

and described as if they were. This was central to the approach to 'map' the precise spatial arrangement of zooids in relation to the colony. Designations identifying internodes defined their vertical and horizontal position, relative to each other, and within the colony. They did not include the three dimensional aspect of colony structure, although of course the spatial arrangement of internodes in relation to one another is ultimately related to this. This aspect was not easily quantified but will be considered qualitatively.

Internodes were designated as follows:-

- Their vertical position (internode generation) was designated by a letter. The first generation as 'A', and so on.
- Their horizontal position within a generation was designated numerically, viewed frontally, and reading from left to right. (For each internode to be accurately related to one another, both internodes which had developed and positions where they had not, were numbered).
- Internodes were differentiated into 'stem', 'S', or 'branch', 'B' types.
- Internodes were differentiated on the basis of whether they were complete, 'C', or incomplete, 'I', depending on whether or not they had bifurcated.

The above facilitated the theoretical spatial reconstruction, in two dimensions, of all of the internodes recorded, including internode length, whether the internode was a 'stem' or a 'branch', and whether it was complete or incomplete.

Autozooids were designated in two respects which provided complementary information, as follows:-

- Each autozooid within an internode was designated with an autozooid number, the most proximal being number one, and so on, to the most distal, the apical autozooid. With the exception of this last, autozooid number also indicated in which of the two series of autozooids within the internode it occurred, relative to the preceding bifurcation. Odd-numbered autozooids are always in the external of the two series and even-numbered in the internal.

(There was a complication in this regard, in respect of vibracula, which will be dealt with when necessary).

- Each autozooid was also designated with reference to its position within an internode in relation to the bifurcation. 'Apical' autozooids, 'A'; the two autozooids immediately proximal to it, 'sub-apical', 'S/A'; and all other autozooids, 'proximal', 'P'. (Positions were calculated back from the apical autozooid i.e. autozooids one and two in an internode of three were designated 'sub-apical'. If the first two autozooids of an internode needed to be selected, this could be done using autozooid number).

The above provided a firm basis for designating the occurrence of polymorphs, since they could be related to the precise autozooid from which they emanated. These in turn could be placed within their internode, and in relation to its length and character, and within the colony as a whole. The only exception to this, for the species in this study, was the axial vibracula, which occurred with absolute constancy in the axils of bifurcations in *S. reptans.*

The designations for polymorphs related simply to their presence or absence. Any variations in usage to accommodate aspects peculiar to a particular polymorph, will be indicated where they occur.

The coded recording scheme described above identified the 'generation' of each internode and whether it was a 'stem' or a 'branch'. At each bifurcation one of the succeeding internodes, the 'stem' internode, deviating only slightly from its predecessor, can be considered as a continuation of the existing 'stem sequence', whilst the second, the 'branch' internode, deviating at a greater angle, can be considered as the first internode of a new 'stem sequence'. It was therefore possible, although all bifurcations occurred in the same way, to differentiate 'stem sequences' on the basis of the number of internodes within them and on the generation of their initial internode. It was also possible to identify the position of each internode within its 'stem sequence'.

Progressing distally along a 'stem sequence' each bifurcation is a mirror image of the one which preceded it, with 'branch' internodes occurring alternately to left and

right. As a result of this and the fact that all internodes are numbered within their generation as if none were missing, it is possible to identify the constituent internodes of any 'stem sequence', generation on generation, using a very simple formula:-

All 'stem sequences' of internodes proceed, from one generation to the next, by a doubling of internode number, alternating with a doubling minus one. An odd-numbered internode in one generation is followed by an internode of double that number in the next, and double that, minus one in the next, and so on.

Apart from the information relating to 'stem sequences', all details were observed through the microscope, recorded on data sheets and then transferred to the computer.

For the detailed study of *S. reptans* it was necessary, for the unpredictably occurring polymorphs, to sub-sample, and separate data sheets were used. This was not necessary for the detailed study of *T. inopinata.*

There had to be a way of recording 'no information', where damage or overgrowth prevented accurate observation. Also, because I was anxious to obtain as much information as possible, I included that relating to 'incomplete' internodes, and it was not possible to designate the position within such internodes of the more distal autozooids. No information in such cases was designated with an 'x'.

Further I separated the 'no information' cases, referred to above, from those cases where there was incomplete development. This was to enable me to distinguish branch tips, which were apparently still growing when the colony was collected, from those which were not. This information was necessary if any investigation into the temporal aspect of polymorph production was to be attempted, using the extent of their development in relation to the growing tip of the internode. No information in such cases was designated with an 'i'.

4.4 POSSIBLE APPROACHES TO USING A CODED RECORDING SCHEME

Ideally one would 'map' entire colonies, and would have a complete picture of all of the relevant details but this was not a practical proposition for even a single colony of any size. There was a need to investigate colonies very extensively since I had had no idea on what scale any patterns of occurrence might occur. Conversely it was also desirable to investigate as wide a range of material as possible to ascertain if there was inter-colony, or perhaps inter-population variation.

In any event, apart from very small colonies, complete mapping for the very small polymorphs was not a practical proposition, and it was necessary to ascertain regions of a colony which constituted genuinely representative sub-samples. This, while reducing the amount of data recorded, could neither compromise the validity of the data obtained, nor reduce the number or variety of investigations possible.

It was important, however extensive the material investigated, that all was kept and identified (i.e., labelled so that a reconstruction of a sample or the entire colony would be possible). Only then, would any possibly necessary re-investigations be possible.

Various approaches were possible, each with their own advantages and disadvantages, and which could be used in a variety of combinations.

Some of the possibilities were:-

- To initially undertake investigations that were believed to be relatively simple or constant. The frontal avicularia of *S. reptans*, on preliminary investigation, seemed to occur simply in relation to the number of the autozooid within an internode. It was straightforward to establish if this was so and to obviate the need for any further investigation.
- To map completely (internodes, autozooids, polymorphs and ovicells), within the main 'stem sequence', from ancestrula to colony edge. This could give

some general indication of polymorph occurrence and any proximal to distal trends.

- To investigate a number of such main 'stem sequences', which could provide some idea of the extent of any variation between colonies and or populations.
- To map an entire colony, if not too large, in respect of internode arrangement and lengths, which could show if any trends were apparent, vertically or laterally, or any overall pattern to colony structure.
- To map ovicell distribution for a complete colony, perhaps in conjunction with the above.
- To sample certain areas of a colony or investigate the occurrence of various polymorphs or ovicells in relation to a number of single parameters. Such an approach would not however allow such factors to be separated from others.
- To carry out a number of straightforward investigations of a single aspect in which colonies would be broken up, the particular aspect studied and recorded, and the material discarded. Such investigations would ignore all other aspects and no follow-up study would be possible. Such investigations could be very useful perhaps in expanding the sample size in respect of findings obtained from a small sample.

There was a need, in relation to polymorph occurrence, to establish regions of a colony which could be used as genuinely representative samples of the colony. It was difficult to see how such could be identified without a very thorough examination of a substantial part of a colony.

The approach investigating a number of main 'stem sequences' seemed initially the most useful, if only because it could establish the extent of variation between colonies and, if desired, populations; and therefore the extent of the need for replicates. Also it would provide basic information, within these 'stem sequences', on internode lengths and polymorph occurrence and distribution. It could well provide information which would suggest simple follow-up studies and perhaps rule out unnecessary investigations.

4.5 THE APPROACHES UTILISED IN CHAPTERS 5 AND 6

4.5.1 Introduction

Whilst ideally all relevant information would be recorded for all of the material investigated, this was not a practical proposition. Any sub-sampling, however, had to be done carefully to ensure its validity, and ideally carried out only when there were reasonable grounds for believing the sample to be representative of the whole. Where this was not the case, it was important to be aware of the factors which were not considered or were outside the scope of the method used.

The use of the coded recording scheme facilitated the investigation, via the computer, of all of the various parameters, both singly and in whatever combinations were required. In order that comparisons could be made it was important that both 'presence' and 'absence' were recorded; and that sites at which a polymorph either could not occur or where, for whatever reason, there was no information were also identified and excluded. The investigation thus resulted in numerous data tables in which the results were expressed as 'percentage occurrences'. This allowed comparisons to be made in terms of relative levels of occurrence in simple numerical terms. Data tables have been used, rather than more arresting bar charts, because the numbers involved frequently varied considerably in respect of different elements of a comparison, and tables are quite explicit in this respect.

There was one polymorph of *S. reptans* which posed a problem using the above approach, the axial vibraculum. The species produces one vibraculum in the axil, and this is produced by autozooid 'f' at the bifurcation, autozooid No. 2 in the 'stem' internode following the bifurcation (Santagata and Banta, 1996 in respect of *S. ferox*). Given that such vibracula are never produced by autozooid 'g', No. 2 in the 'branch' internode, I had to decide how to treat this information, whether to ignore it as 'could not occur' or as 'absence'. I decided to ignore it and to treat the vibracula which occurred simply as 'axillary', which describes their actual location. (They also differ from all other vibracula in not giving rise to a potential rhizoid). I did not then include them in the analysis in respect of the parameters used elsewhere. They

occurred on all No. 2 autozooids in 'stem' internodes and, on essentially an identical number of such autozooids in 'branch' internodes, they were absent.

4.5.2 Statistics

The data were not normally distributed and only non-parametric tests of significance were possible. Chi-squared tests were carried out, and where these involved 2 x 2 tables, Yates' Correction for Continuity was applied. For some of the larger tables the results were statistically significant, but the assumption that the minimum expected cell frequency should be five or greater (or at least 80% of cells have expected frequencies of five or more) (Pallant, 2001) was not satisfied. This, depending on its extent, clearly reduced the value of the result, but to a varying degree. Where this was the case, I have therefore, in addition to the Chi-Square value and the Significance level, detailed the extent of this failure by two numbers in parentheses separated by a '/' indicating the number of such cells and their percentage of the total, respectively.

In relation to the above, I believe that especially in respect of the spatial arrangement of polymorphs, whose observed patterns of occurrence, I suspect, often have no single cause, it is unwise to consider these parameters in strict isolation. The relative frequencies are perhaps composite results, but they are of course, all that we have.

4.5.3 Preliminary study

Scrupocellaria reptans was the first species to be investigated (Chapter 5) and the preliminary investigation (Section 5.2) used sampling which inevitably had no previously established justification. The main 'stem sequences', all of the 'stem' internodes from the very first of the colony to its edge, of 30 colonies from two separate populations, were investigated in respect of the lengths and sequence of their constituent internodes. The numbers and precise location of the various polymorphs were also recorded for five main 'stem sequences' of each population. This was a preliminary investigation, which aimed to address a central concern, the extent of any inter-colony or inter-population, variation. Colonies investigated were from a littoral

population at Musselwick, Milford Haven, Pembrokeshire, and a sub-littoral population at Bay Fine off the SW of the Isle of Man.

There was no *a priori* reason to suppose that main 'stem sequences' actually existed, it was simply a sampling method to include zooids from each generation of internodes of a colony. The approach ignored the possibility that the characteristics of the main 'stem sequence' might not also obtain in other areas of the colony, and other aspects of the spatial arrangement of zooids within the colony would remain unrecorded. It was felt, however, that such an approach would provide a useful starting point in quantifying levels of variation, and that it would, perhaps, rule out the need for certain other work and perhaps suggest areas where further work was likely to be most productive.

The preliminary study gave no indication of marked variation between colonies, or populations, in respect of internode lengths. It did suggest, however, since it was apparent that the length of the constituent internodes of these main 'stem sequences' was very different from that of the complete colonies, that there was some cryptic organisation of different length internodes, within a colony, worthy of investigation.

In these main 'stem sequences', five from each population, the occurrence of the majority of polymorphs was remarkably constant. Only in respect of lateral avicularia was there evidence of inter-colony and inter-population variation.

As a result of the above, it was decided to carry out a detailed study of a single colony from the Musselwick population (Section 5.3).

4.5.4 Detailed studies

4.5.4.1 *SCRUPOCELLARIA REPTANS*

It is impossible, because of the three-dimensional nature of a complete colony, to manipulate it under a microscope sufficiently well to record the characteristics of interest throughout the entire colony, as damage is inevitably caused in the process. It was necessary to divide the colony into a number of pieces, which were small

enough to be looked at in their entirety, with minimal damage to them. These pieces were stored separately, each together with a label recording the coding scheme designation (the generation, and the lateral position within it) of the most proximal internode at its base. Each portion of the colony was examined as a separate entity but the information recorded was in relation to the colony as a whole.

Overall colony form is variable and in some cases, especially those colonies where there are physical obstructions to colony growth, appears to be very much determined by the shape of the space available to it. Because of this, a large colony, without any obvious obstructions to growth, was chosen, in an attempt to get some purchase on potential colony size and form, affected as little as possible by any physical constraints of available space. It would also include any features, which might develop late in the astogenetic process. A vast amount of data would be involved if the complete colony was to be mapped, and it was necessary to try to reduce this, without compromising the validity of the data. The problem was much more acute in respect of the heterozooids than the autozooids. The size of the samples selected within a colony needed to reflect this.

A preliminary investigation established that the extent of growth was uneven, with regions of limited and very extensive growth. There was no evidence to suggest that if the colony was divided vertically down an imaginary central axis, one half would differ appreciably from the other. Indeed, it suggested that any lateral variation would be on a somewhat smaller scale.

I decided that the scale and nature of any lateral variation would become apparent if I recorded all the information relating to one half of the colony. This approach made possible the investigation of a whole range of aspects, features, trends and correlations as will be shown below, but it must be borne in mind that the data refer strictly to only half a colony. It is of course, once a characteristic becomes apparent, a comparatively simple matter to check whether or not it is also present in other colonies of the same, or indeed other populations.

The preliminary investigation had highlighted the considerable difference in the percentage occurrence of the different internode lengths in the main 'stem sequences' and the colonies as a whole.

This finding posed the questions:-

- If there was a main 'stem sequence' distinguished by the lengths of its internodes, were there also other 'stem sequences', with this or another particular characteristic?
- If so, how were such 'stem sequences' arranged within the colony?
- If this was the case, how were all other internodes, within 'stem sequences', arranged relative to them?

'Stem sequences' were an unforeseen element which required recording, and the coded recording scheme, formulated in advance, did not facilitate this. In any event, it would have been very difficult to record the relevant information, much of which was cumulative, in conjunction with the directly observable characteristics. Details of the lengths of 'stem sequences', their generation of origin, and the numerical position of internodes within them, were established manually and incorporated subsequently into the datasheets on the computer.

This revealed a limited number of very long 'stem sequences', with the internode composition of the main 'stem sequences' of the preliminary study, and a great number of short 'stem sequences' with a somewhat different internode composition.

It was known that the internode composition of the colony and the 10 longest 'stem sequences' differed in two respects; the proportions of the internodes of the various generations, and that of 'stem' and 'branch' internodes. It was therefore necessary to establish whether or not these differences could account for those observed between the colony and the 10 longest 'stem sequences'.

It was also apparent that the long 'stem sequences' were laterally well spaced. It was difficult to represent clearly, diagrammatically, the spatial arrangement of internodes, even within a limited region of the colony, because the numbers involved are so

large. Few attempts have been made to represent the spatial arrangement of different length internodes within a colony. A very early system of notation denoting such characteristics, and the positions of gonozooids, for crisiids, was developed by Smitt (1865) and modified by Harmer (1891). It was complex system, not a practical proposition for extensive material, and did not give a straightforward visual impression of spatial relationships, but did show what needed to be done.

Several different approaches have been adopted in this study, each illustrating certain aspects well whilst distorting others. The fact that each internode had a unique designation, which defined both the generation of internodes in which it occurred, and its lateral position within it, did however; enable a number of parameters to be described.

A diagrammatic representation of the number and location of those internodes that did occur, in relation to all of the possible positions in which they were possible, Figure 5.4, was made. This, whilst grossly distorting the picture from one generation to the next, did establish that beyond the proximal region of the colony, where virtually all the internodes possible did develop, as the colony developed internodes were essentially, and increasingly, restricted to narrow groups, each centred on a long 'stem sequence'.

It was then possible to diagrammatically represent all of the internodes developing beyond the proximal region, from a single long 'stem sequence', with some confidence that the 'aggregations' associated with other long 'stem sequences' would be similar (Figure 5.5).

I felt that I now had a handle on the pattern of lateral variation within the colony and that, for unpredictably occurring polymorphs, an 'aggregation' of internodes, constituted a natural sub-sample.

As far as polymorphic heterozooids were concerned, the limited evidence of the preliminary study suggested certain consistent characteristics and some less regular trends. For lateral avicularia, there was evidence of variation between colonies and

populations. There were a number of aspects to be considered in any attempt to describe the pattern of distribution of a particular polymorph. It must always be borne in mind that the pattern of distribution of a polymorph within a colony may appear without order simply because one or more factors involved in its causation are not readily apparent. Environmental factors may be involved, the influence of which, singly or perhaps synergistically, may vary temporally. Having said that, I believe that much can be said about the pattern of occurrence of various polymorphs within a colony, providing that all of the relevant parameters and their possible interactions are considered. Whilst the spatial arrangement of polymorphs probably results from several different factors, in the first instance, all that can be recorded is the actual spatial arrangement that has resulted. The results were investigated and revealed that in addition to a genuinely spatial dimension, polymorphs probably interacted with each other; and also, that the level of occurrence could influence a pattern of distribution. Subsequently, it was sometimes possible to extract some pointers as to how the various factors involved, had interacted to produce the observed result.

Some polymorphs (spines and scuta) occurred constantly on all autozooids or in all branch axils (axial vibracula); another (frontal avicularia) occurred absolutely predictably on only certain numbered autozooids within an internode. For these polymorphs there was no other aspect to their pattern of occurrence.

For those polymorphs which occurred unpredictably (lateral avicularia, vibracula and rhizoids), and which are very time-consuming to record, it was always going to be necessary to sub-sample. The difficulty had been in determining a region of the colony which would be representative of the whole. The fact that internodes, within this colony, were arranged, as the colony developed, in an increasingly clumped manner around a small number of long 'stem sequences', indicated regions which constituted natural sub-samples.

In order to include the distal-most parts of the colony it was desirable to use the longest of the long 'stem sequences', the internodes of which are shown in Figure 5.5. Internodes essentially formed a vertical, lanceolate, band, centred on a long 'stem sequence', with very infrequent 'arms', to either side. I still needed to reduce

the amount of material investigated, if this was possible without compromising the validity of the data. I decided that any vertical variation, if it existed, would probably be continuous and that it would not be necessary to record all of the generations. Similarly, any lateral variation which occurred would be likely to occur in relation to a long 'stem sequence' and that taking all of the internodes within it, together with all of those which developed to one side, would be sufficient.

A complication became apparent regarding the actual location of vibracula which occurred on No. 1 autozooids:-

Their much higher level of occurrence on autozooid No. 1, relative to all other odd-numbered autozooids, in all of the material investigated was initially as puzzling as it was consistent. The explanation may be in the following:-

The fact that the joints between internodes cut across the proximal ends of both number one autozooids, in the two internodes resulting from a bifurcation, creates a complication, which must be allowed for, in respect of such vibracula, in translating autozooid number into internal/external, autozooid series. Following a bifurcation, the vibracula on both new number one autozooids occur at the distal end of the preceding internode, one in its internal, and one in its external series of autozooids, although both of these autozooids are predominantly in the external series of the new internodes.

Vibracula on No. 1 autozooids were, therefore, in terms of autozooid series, 'reallocated' to the actual series in which they occurred. This was a simple exercise due to the consistency of the branching pattern and, as a result of the numerical arrangement of stem and branch internodes within a generation of internodes. As a consequence of this, the vibracula on No. 1 autozooids of stem internodes occurred in the internal series of the preceding internode, and those of branch internodes in the external.

4.5.4.2 *TRICELLARIA INOPINATA*

The detailed investigation into the spatial arrangement of zooids within a colony of *Tricellaria inopinata* (Chapter 6) utilised the same methodology, centred on the coded recording scheme. The fact that the detailed study in respect of *S. reptans* had been carried out on ~50% of a very large colony suggested that it would be worthwhile, for this species, to use a complete — if rather smaller — colony.

Differences between the two species meant that this investigation was different in certain respects. There are no frontal avicularia or vibracula in *T. inopinata,* lateral avicularia occur in a range of sizes rather than 'small' and 'giant' and these needed to be recorded in a limited, but genuinely representative region of the colony. If the colony structure, in terms of its 'stem sequences', was similar to that of *S. reptans* this would be in relation to 'aggregations' of internodes associated with long 'stem sequences'.

Certain mural spines and the scuta, both constant in morphology in *S. reptans,* exhibited variation in *T. inopinata.* Certain spines were sometimes bifid and scuta exhibited a great range of size and morphology. It would have been desirable to undertake an investigation into any possible relationship between variations of these two polymorphs and also, perhaps, the presence or absence of ovicells. However, such a study required very pristine material; spines and scuta are very easily damaged and no quantitative study was possible.

4.5.5 Supplementary studies

Although the two detailed studies were felt to be the most useful approach, because only they could investigate any colony wide aspects of zooid occurrence, they were not in all respects completely satisfactory.

Firstly, there was the matter of the constancy of characteristics throughout the species. The preliminary investigation of main 'stem sequences' of 15 colonies from each of two separate populations of *S. reptans*, established the presence of long 'stem sequences' with a particular internode composition, as a consistent characteristic.

For the spines, scuta, frontal avicularia, vibracula and rhizoids, there was no evidence of inter-colony or inter-population variation. Lateral avicularia however, occurred five times more frequently in the material from the I.O.M. and there was evidence of inter-population variation at Musselwick.

Secondly, in some instances, tentative conclusions had necessarily been drawn from too small a sample, notably for ovicells in *S. reptans.* In respect of *T. inopinata,* the numbers and lengths of 'stem sequences', in the partly developed colony was, as a result, less conclusive than it might have been. The presence or absence of lateral avicularia and ovicells, within long 'stem sequences' of *T. inopinata,* also invited further study.

It was therefore, desirable to carry out a number of supplementary studies, although these regrettably did not always include colony-wide aspects of zooid distribution. Nevertheless such studies acted as useful crosschecks.

For *S. reptans* there was evidence that, for lateral avicularia, there was considerable variation between the two populations. Although this was only one polymorph, it was only two populations! It would obviously be worthwhile to look at material from another geographically separate population. I used material from Swanage in Dorset.

4.5.5.1 *SCRUPOCELLARIA REPTANS*

4.5.5.1.1 Irregularly occurring polymorphs

In an enquiry designed to look further at possible inter-colony or inter-population variation, a supplementary study was carried out on an 'aggregation' of internodes in association with its long 'stem sequence'. Data were recorded using the coded recording scheme (the raw data are in Appendix 'H')

4.5.5.1.2 Possible delayed development of lateral avicularia, vibracula and rhizoids

The variations in the form of scuta, related to their ontogenetic stage of development, are very obvious. They can be used to distinguish branch tips which were apparently still growing when the colony was collected from those which had suffered damage. Examination of unbroken branch tips and the minimum distance each of the three polymorphs occurred from them, established the extent of any obligatory delayed development.

4.5.5.1.3 Ovicell occurrence related to autozooid number and the presence/absence of frontal avicularia

Because ovicells were only sparsely present in the large colony studied in Section 5.3, I felt that a limited investigation into the relationship between autozooid number (and therefore frontal avicularia occurrence) and ovicell production would be worthwhile. A large fertile colony, collected at Swanage was selected, broken up into manageable size pieces, and 300 randomly selected internodes, each with at least one ovicell, were investigated, simply relating ovicell occurrence to autozooid number, and thus to frontal avicularia occurrence, (see Section 5.4.4). (Raw data in Appendix 'I').

4.5.5.2 *TRICELLARIA INOPINATA*

4.5.5.2.1 The frequency of occurrence of different lengths of 'stem sequences', and internode lengths in relation to their position within them

A substantial portion of a large colony was investigated solely in respect of the number and lengths of its 'stem sequences', and their constituent internodes.

4.5.5.2.2 Ovicell occurrence related to the presence/absence of lateral avicularia in long 'stem sequences'

In the detailed study of *T. inopinata,* ovicells were virtually absent from the four long 'stem sequences'. Although numbers were very small, they offered some evidence supporting the idea that lateral avicularia occurrence could be related to the presence or absence of ovicells. A single colony has only a small number of long 'stem

sequences', and it was considered worthwhile to investigate a number of long 'stem sequences' from several colonies, in this respect.

4.5.5.2.3 The distribution of internodes, lateral avicularia and ovicells, within two long 'stem sequences', and all of the internodes which developed from them

A supplementary investigation was deemed necessary to include two aspects not included within the detailed study. Firstly, no attempt had been made to relate internode length, or the spatial arrangement of internodes relative to each other or to the overall pattern of occurrence of lateral avicularia and ovicells. Secondly, the fact that lateral avicularia occurred in different sizes had been ignored. The data relating various parameters in respect of lateral avicularia and ovicell occurrence in the detailed investigation was from one colony, and it was worthwhile to repeat this, in respect of material from other colonies. Two long 'stem sequences', together with all of the internodes which developed from them, were chosen from two different colonies, and all of the relevant details recorded using the coded recording scheme. Because, in one instance, the material selected was not from a complete colony, I did not know the actual generations of the internodes, hence they were designated from the most proximal, 'X', 'X' + 1.... (Raw data in Appendices 'L' and 'M').

4.5.6 Unquantified investigations

Two aspects of the approach adopted to this study have mitigated against both the amount and range of material investigated:-

- Firstly, there was the need to investigate as much of a single colony as possible in order that any pattern of occurrence, on however large a scale, was apparent.
- Secondly, although the heterozooids were often small and difficult to see, it was decided to record as far as possible their definite presence or absence. This, although time consuming, was felt to be worthwhile, because it enabled true percentage occurrence figures to be obtained.

Ideally sample sizes would have been larger, and the range of material, number of colonies, and perhaps populations, would have been greater. The supplementary studies described above, were carried out to remedy, to some degree, the inevitable deficiencies of the approach adopted. Further efforts were made, in the form of unquantified investigations, which tackled particular concerns remaining after the supplementary studies.

Although not ideal, the use of unrecorded investigations concentrated attention on the areas where it was most needed. Additional material was examined, with particular attention to:-

- Features which were important, but which had been observed in only a limited amount of material.
- Situations in which the level of a polymorph's occurrence varied between colonies of two populations.

The approach of this study has been to investigate how zooids are arranged relative to one another within a colony. The carrying out of replicate studies in just three populations of *S. reptans* highlighted substantial variations in the level of occurrence of two polymorphs and species wide generalisations should be approached with caution. Nevertheless, conversely, there was much evidence of consistency in respect of many characteristics, often in both species, and variations in level of occurrence blunted rather than obliterated patterns previously observed.

CHAPTER 5 – THE SPATIAL ARRANGEMENT, WITHIN A COLONY, OF THE AUTOZOOIDS AND HETEROZOOIDS OF *SCRUPOCELLARIA REPTANS*

5.1 INTRODUCTION

Before defining the objectives of this study, it is necessary to give a conventional morphological description of the species. This will provide a background before I indicate, in the 'Objectives' section below, the limitations, as I see them, of such a description, and suggest some of the questions which could be asked, to produce a more comprehensive picture of a colony as an entity.

Arborescent colonies of cellularine bryozoans, within the family Candidae, exhibit a general constancy of colony form (Harmer, 1923). They consist of unilaminar, generally biserial branches, internodes, which consist of various numbers of autozooids. Internodes, on completing their growth, generally bifurcate to produce two new internodes. Polymorphic heterozooids, fulfilling various functions, are numerous and varied throughout the family.

Although Harmer (1923) defined various bifurcation types as a useful tool for separating genera, Canu and Bassler (1929) still felt that this was achieved principally on the basis of heterozooecia, although they recognised that various exceptions resulted in different interpretations and conclusions. Today the various genera within the family are essentially differentiated in respect of two separate criteria (Ryland, 1965):-

- The spatial relationship between the autozooids in the region of the bifurcation, in their relation to it, and to the joints which occur after it.
- The presence or absence, number, morphology and distribution of the various polymorphs.

There remains, however, much variation in respect of the latter within a genus.

Polymorph occurrence, morphology and distribution, vary considerably at all taxonomic levels within the Bryozoa. Basic polymorph types themselves may be monomorphic or polymorphic. For *S. reptans,* as with many other Bryozoa, the architecture of the colony prevents certain polymorphs occurring in certain positions. Some polymorphs occur absolutely constantly, on all autozooids, or at certain locations within the colony, but many occur with varying degrees of unpredictability. Of the latter, some are clearly concentrated in certain areas of the colony, but for many, their pattern of occurrence, if they have them, are not readily apparent. It is conceivable that the presence of a certain polymorph is positively or negatively correlated with that of a second polymorph, or with embryo and ovicell production. It is also quite possible that polymorph occurrence may be inhibited, or stimulated, by environmental factors, acting singly or synergistically, the effect of whose influence may also vary temporally; and result in no obvious level, or pattern, of occurrence.

5.1.1 Characteristics of the family Candidae d'Orbigny, 1851

Colonies are arborescent, with unilaminar branches, generally biserial, in which case the two rows of autozooids are staggered relative to one another, with a centrally positioned autozooid wedged distally between the distalmost pair. Internodes bifurcate asymmetrically at intervals, and these generally involve flexible chitinous joints between internodes. The precise arrangement of autozooids, in relation to these joints and the bifurcation, is important in distinguishing genera within the family. Autozooids lightly calcified with an extensive frontal membrane. Mural spines generally present. A specialised modified spine, the scutum, which is unique to the family, is often present. This, varying in shape and extent, overarches the frontal membrane. Avicularia and vibracula are generally present and the colony is anchored to the substratum by tubular rhizoids. Embryos are brooded in ovicells, which are sub-globular and hyperstomial.

5.1.2 Characteristics of the genus *Scrupocellaria* van Beneden, 1845

Colony form is erect/procumbent, and branching. The branches, each of which is jointed at its inception, consist of two rows of autozooids, staggered relative to each other, and with a centrally placed distal autozooid proximal to each bifurcation. The arrangement of autozooids to each other and to the joints at bifurcations, accords with Type 8 of Harmer's (1923) classification (see Fig 5.1, below). Autozooids narrow proximally, having an oval frontal membrane; mural spines generally present, as is the scutum, which overarches the frontal membrane. The ancestrula is vase shaped with mural spines; it and the developing colony are attached to the substratum by tubular rhizoids. Avicularia and vibracula are found in most species but not on all autozooids. Distally sited lateral avicularia are generally present, and proximally sited frontal avicularia less frequently so, sometimes only in association with ovicells. Baso-distally sited vibracula generally present, and one or two may be present in the axil of bifurcations. Ovicells subglobular and hyperstomial (Hayward and Ryland, 1998).

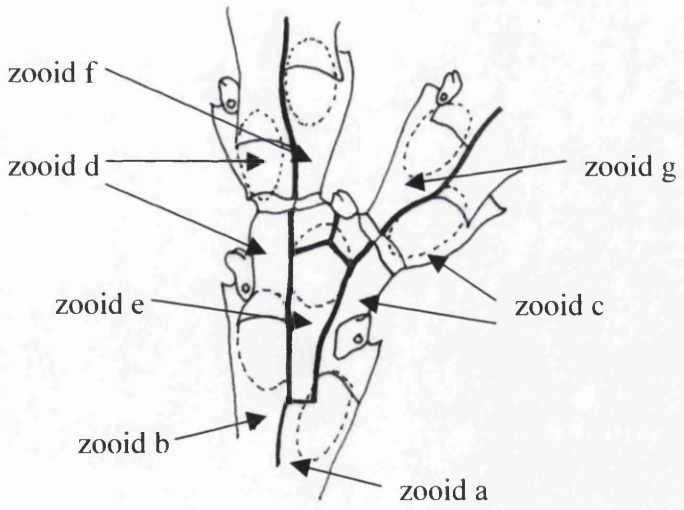


Figure 5.1 The arrangement of autozooids relative to each other, and to a bifurcation in Harmer's bifurcation type 8 (modified after Hayward and Ryland)

5.1.3 A general morphological description of *Scrupocellaria reptans* (L.)

Scrupocellaria reptans has been described in numerous regional faunas (Hincks, 1880; Prenant and Bobin, 1966; Hayward and Ryland, 1998). Lutaud (1953) provided a detailed study of the species, with particular attention to the ancestrula, and the growth of young colonies. The description below essentially summarises the position prior to this investigation, although there is no absolute consensus, even among recent accounts, in regard to internode length; and, in respect of polymorph occurrence and distribution, information is generally qualitative.

5.1.3.1 THE COLONY

A well-developed colony of *S. reptans* has the overall appearance of a miniature, deciduous bush in winter. When spread out under water, colonies may be ~25mm high and ~35mm in diameter. Such a colony consists of a great number of branches, internodes, each of which consists of a number of autozooids. The entire colony originates from one such internode, which arises from the first autozooid of the colony, the ancestrula. This internode bifurcates at its distal end to produce two further internodes, which in their turn, at their distal ends each produce two more, and so the colony grows distally and laterally.

5.1.3.2 INTERNODES AND BIFURCATIONS

Internodes are unilaminar, consisting of a single layer of autozooids, all with their lophophores projecting on the same side, and vary in length with the number of autozooids they contain, although there is no consensus in the literature as to the actual numbers or their range. (Hincks, 1880, 'five or seven'; Ryland, 1965, "five to seven mostly"; Prenant and Bobin, 1966, 'five to seven'; Hayward and Ryland, 1998, "five to eight but up to fourteen"). Lutaud (1953) 'internodes most frequently of five, seven or nine autozooids', stated that all complete internodes were of an odd number of autozooids.

Autozooids within an internode are essentially arranged biserially but with the two rows, staggered slightly relative to one another, and with a single centrally positioned

'apical' autozooid, wedged between the distal ends of the distalmost pair. The 'sub-apical' pair of autozooids, one of which is slightly distal to the other, produce, via distal buds, the first autozooids of the external rows of autozooids of the new internodes. Hence the asymmetrical nature of bifurcations. The apical autozooid produces, via a distal bud, the first autozooid of the internal row of one of the new internodes, the primary ramus, and via a disto-lateral bud, the first autozooid of the internal row of autozooids of the other, the secondary ramus. Because the apical autozooid is always distal of the sub-apical pair, the internal rows of both new internodes are always slightly distal to the external. One internode, the secondary ramus, originates slightly more proximally, and diverges at a greater angle, and the other, the primary ramus, originates slightly more distally, and diverges less from the direction of the internode which gave rise to it. Because the more distal of the sub-apical pair of autozooids of one internode develops, at its bifurcation, into an internode in which the more distal of the sub-apical pair of autozooids is on the opposite side, an alternation in the handing of successive bifurcations is inevitable (Lutaud, 1953).

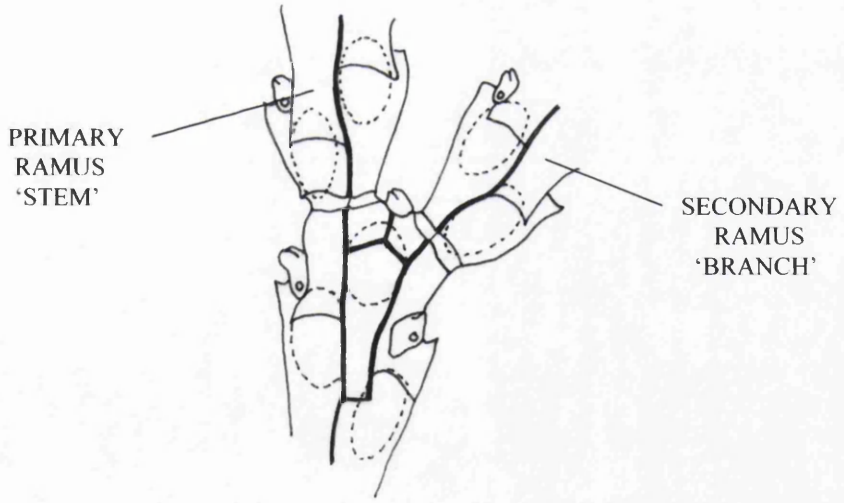


Figure 5.2 Primary and secondary rami, 'stem' and 'branch' internodes, following a bifurcation (modified after Hayward and Ryland)

The joints between internodes consist of two echelons of chitinous tubes, within the proximal regions of the first two autozooids; and when these are fully formed, a narrow band of calcification dissolves away, leaving the chitinous tubes as a strong, but flexible joint, between one internode and the next. Harmer (1923) provided a comprehensive account of the process.

Lutaud (1953) described and figured the consistent branching pattern which obtains throughout the colony.

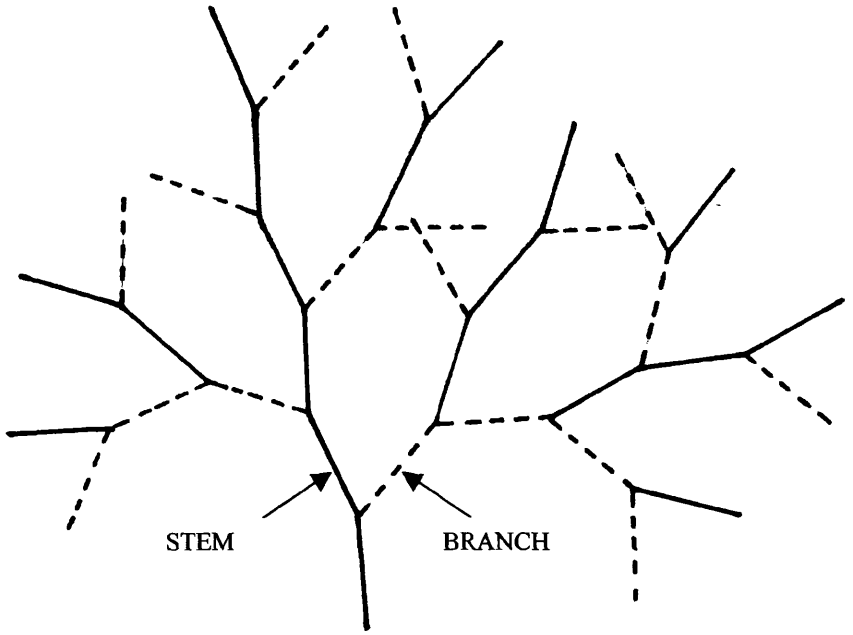


Figure 5.3 The consistent pattern of 'stem' and 'branch' internode production at bifurcations (modified after Lutaud)

Lutaud recognised that one element of this consistent branching pattern, the fact that each bifurcation was a mirror image of its predecessor, resulted in what she termed, the "axiale principale", maintaining an essentially constant direction of growth.

5.1.3.3 AUTOZOOIDS

The autozooids within an internode are not all identical in size and shape. The apical autozooid is truncated proximally, where it is wedged in between its predecessors, and the first two autozooids are attenuated proximally, although not equally so, as they span the joint between the two internodes. All other autozooids are longer than the former and shorter than the latter. All autozooids, except the ancestrula, the very first autozooid of the colony, which results from the metamorphosis of the originally planktonic larva, are, apart from the differences referred to above, identical in form. They are essentially rhombic, narrowing proximally, with an oval frontal membrane occupying the most distal two-thirds, and a smooth gymnocyst occupying the most

proximal one-third. The ancestrula is bath shaped with mural spines. The associated polymorphs, which occur on some or all autozooids, are described below.

5.1.3.4 HETEROZOOIDS

5.1.3.4.1 Simple straight spines

Simple straight spines occur around the distal end of the rim surrounding the frontal membrane, generally three on the outer and two on the inner edge. They occur in similar fashion on all autozooids, except that there are left and right 'handed', and central, assemblies. The ancestrula has nine spines symmetrically arranged around its more extensive frontal membrane (Lutaud, 1953).

5.1.3.4.2 Scuta

Scuta are produced from the mid point of the internal edge of the rim surrounding the frontal membrane, on all autozooids except the ancestrula. When fully developed, they form a cervicorn structure, which covers much of the frontal membrane.

5.1.3.4.3 Frontal avicularia

Large frontal avicularia occur on the frontal wall, just proximal to the frontal membrane. They do not occur on all autozooids, there are often two on an internode of five autozooids and three on an internode of seven (Hincks, 1880).

5.1.3.4.4 Lateral avicularia

Lateral avicularia vary little in morphology. They are triangular, with the mandible generally directed laterally. The majority are very small, often difficult to see, as they may be hidden behind the spines, but much larger giant forms, do occur infrequently. Lateral avicularia occur disto-laterally, in relation to the autozooid from which they develop. They cannot occur on the apical autozooid of each internode, as this has no external lateral edge, and they are frequently missing from other autozooids.

5.1.3.4.5 Vibracula

Vibracula are sited laterally, proximally, basally, in relation to the autozooid from which they arise. They emanate from either the distal half of a double-tiered chamber of a single polymorph, as is generally claimed (Hincks, 1880; Harmer, 1923; Ryland, 1965), or perhaps, from the more distal of two separate chambers (polymorphs) (Santagata and Banta, 1996). Banta has confirmed to me (pers. com.) that the two are separate zooids. Whichever is the case, the distal chamber/polymorph always gives rise to a vibraculum. Vibracula also occur in the axil of each bifurcation, from a single chamber/polymorph and therefore without a rhizoid.

5.1.3.4.6 Rhizoids

Simple tubular rhizoids anchor the colony to the substratum and are concentrated in the proximal region of the colony. Where they occur, they are sited, as are the vibracula, laterally, proximally and basally, in relation to the autozooid from which they arise. They emanate from either the proximal half of a two-tiered chamber, or from the more proximal of two separate chambers (polymorphs), as described above. Whichever is the case, the more proximal may give rise to a rhizoid. A number of workers have observed that the morphology of the distal end of the rhizoid is varied in relation to the nature of the substrate it encounters (Peach, 1877; Hincks, 1880). If this is a flat surface, a structure similar to an algal holdfast, although flatter, develops, which adheres to the substratum. If on the other hand the substrate is such that it requires a more three-dimensional attachment, e.g. a sponge, the rhizoid penetrates the substrate, and anchors itself within by a series of recurved hooks.

5.1.3.5 EMBRYOS AND OVICELLS

Embryos are dull pink, are found throughout the year, but are most numerous from June to October (Hayward and Ryland, 1998). The ovicells, in which they are brooded, are hyperstomial, sub-globular with an ectooecium which has a number of pores. Ovicells in *Scrupocellaria* are produced by the autozooid distal to the one producing the embryo (Nielsen, 1985) and they cannot occur on the apical autozooid

of an internode because there is no autozooid directly distal to it. They are often confined to the autozooids on one side of an internode (Hayward and Ryland, 1998).

5.1.4 Objectives

From the above description it is clear that although much is known about the morphology of the various zooids that make up a colony, information on the arrangement of them within it, beyond Lutaud's identification of the constant branching pattern, is much less evident. I am aware of no attempt to describe the arrangement of internodes within a colony. There is no general agreement within the literature as to the lengths of the internodes; only Lutaud (1953) recognised that all internodes consisted of an odd-number of autozooids. There is only limited information on the presence or absence of polymorphic zooids and their distribution within a colony.

Underlying this investigation is the belief that in many Bryozoa, especially erect forms, the spatial arrangement of all the zooids, both autozooids and heterozooidal polymorphs, within a colony may exhibit characteristics, which could be described with greater definition than is generally the case. In the case of the autozooids this could reveal previously undescribed structure, and for both, it could reveal aspects of colonial organisation previously unsuspected, and perhaps suggest new lines of enquiry.

Questions concerning pattern include:-

- Continuous exponential growth in the number of internodes, generation on generation, is not possible, given the finite space available, and not all internodes can produce two more. Is there an overall pattern to those produced and aborted, and indeed to the form of the colony as a whole?
- Do the various length of internodes occur in any consistent way within the colony?
- Do 'stem' and 'branch' internodes exhibit any different characteristics?
- Do polymorphs exhibit any consistent pattern of occurrence, e.g. in relation to internodes, bifurcations, particular regions of the colony, or the entire colony?

- Are there any correlations, positive or negative, between the occurrence of any two polymorphs?
- Are there any correlations, positive or negative between the occurrence of any polymorph and ovicells?
- Does the distribution of ovicells, and therefore reproductive zooids, exhibit any particular pattern?

Essentially I have mapped, as completely as possible, the occurrence, morphology, and distribution of both auto and heterozooids. The gross arrangement of the former, within a colony, is clearly fundamental to any structure it may possess. There may well also be some cryptic arrangement of various internode lengths and types. The spatial disposition of polymorphs is secondary to that of autozooids, but of great importance in terms of how a colony functions as an entity.

5.2 PRELIMINARY STUDY OF THE MAIN 'STEM SEQUENCES' OF COLONIES COLLECTED LITTORALLY AT MUSSELWICK, PEMBROKESHIRE, AND SUB-LITTORALLY AT BAY FINE, ISLE OF MAN

5.2.1 Introduction

This preliminary investigation was aimed at establishing the arrangement of autozooids and their associated polymorphs, within internodes of a main 'stem sequence', from the first internode, via all successive 'stem' internodes to the edge of the colony. This, since it sampled each generation of internodes, would also provide information on any changes which might occur from one generation to the next. The method could also throw some light on the extent of any inter-colony, or, since colonies from two separate populations were investigated, inter-population, variation. Thirty colonies were investigated, in respect of the internode lengths and their sequence, within the main 'stem sequences'; 15 from a littoral population at Musselwick, Milford Haven, Pembrokeshire, and 15 from a sub-littoral population at Bay Fine, off the south west of the Isle of Man. For five colonies from each population all of the polymorphs associated with them were also recorded.

5.2.2 Results

5.2.2.1 INTERNODE LENGTHS AND THEIR SEQUENCE WITHIN THE MAIN 'STEM SEQUENCES' OF 30 COLONIES FROM TWO POPULATIONS

Did these main 'stem sequences' exhibit any particular characteristics in terms of the lengths and/or sequences of their constituent internodes?

Internode generation	Internode lengths and their sequence														
14											5				
13								5			5				
12								5			5				
11		5						5			5				
10	5	5				5		7			5	5			
9	5	5		5		5		5	5		5	5			
8	5	5		5		5	5	5	5	5	5	5			5
7	5	5		5		5	5	5	5	5	5	5			5
6	5	5	5	5	5	5	5	5	5	7	5	5	5	5	5
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
3	5	5	5	5	5	5	5	5	5	7	5	5	5	5	5
2	5	5	3	5	3	5	3	5	3	5	5	3	3	3	5
1	4	4	4	3	4	4	4	4	4	4	6	4	4	4	4
Colony No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

Internode generation	Internode lengths and their sequence														
14						5				5					5
13	5					5				5				5	5
12	5					5				5				5	5
11	5		5			5				5				5	5
10	5	5	5		5	5			5	5			5	5	5
9	5	5	5	5	5	3	5	5	5	5			5	5	5
8	5	5	5	5	5	5	5	5	5	5		5	5	5	5
7	5	5	5	5	5	5	5	5	5	5		5	5	5	5
6	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5	7	5	5	5	5	5	5
4	5	5	5	5	5	5	5	5	5	5	5	5	3	5	5
3	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
1	4	4	3	4	3	3	3	3	5	4	3	3	3	3	4
Colony No.	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30

Table 5.1 Internode lengths, and their sequence within the main 'stem sequences' of 30 colonies of *Scrupocellaria reptans;* (colonies 1-15 from Pembrokeshire, colonies 16-30 from the Isle of Man)

There is no indication in Table 5.1 (the raw data are in Appendix 'B') that there was any inter-colony or inter-population variation. From the table two interesting characteristics are apparent:-

- Whilst the vast majority of internodes were of an odd number of autozooids, those of the first generation (no ancestrulae present) were sometimes of an even number.
- Although there was no consistent sequence of internode lengths, the first generation of internodes were almost all of three or four autozooids, the second generation almost exclusively of three autozooids, and in all subsequent generations, virtually all internodes were of five, or, very rarely seven, autozooids.

How frequently did the various internode lengths occur?

Autozooids in internode	Frequency	Percentage occurrence
3	33	11.3
4	18	6.1
5	237	80.9
6	1	0.3
7	4	1.4
Total	293	100.0

Table 5.2 The numbers and percentage occurrence in the 30 main 'stem sequences' of the various lengths of complete internodes

Table 5.2 shows that 80% of internodes were of five, and <2% were of seven, autozooids.

Whilst not apparent from Tables 5.1 and 5.2, it was very obvious when viewing the colonies under the microscope, that internodes of seven autozooids were actually very numerous, perhaps the most numerous, in the colonies as a whole, although they were very rare in these main 'stem sequences'. Clearly, different length internodes do exhibit some degree of spatial organization.

5.2.2.2 POLYMORPH OCCURRENCE AND DISTRIBUTION

Five main 'stem sequences' from each population were investigated in respect of polymorph presence or absence. (The raw data are in Appendix 'C'). It is important to remember that all information in this section refers to main 'stem sequences', not complete colonies.

5.2.2.2.1 Simple straight spines

Spines occurred as described in Section 5.1.3.4.1, around the distal end of the frontal membrane in an apparently consistent manner. Naturally these were often broken, frequently at the base. They occurred on all autozooids, of all the main 'stem sequences', of all of the colonies.

5.2.2.2.2 Flattened branched spines (scuta)

Scuta occurred as described in Section 5.1.3.4.2. As with the simple straight spines, left and right-handed forms existed in the two series of autozooids, with that of the apical autozooid being the reverse of the autozooid immediately proximal to it. They occurred on all autozooids, of all the main 'stem sequences', of all the colonies (except the ancestrulae).

5.2.2.2.3 Frontal avicularia

Frontal avicularia occurred on the gymnocyst, just proximal to the frontal membrane. They were always large, and occurred on all odd-numbered autozooids, except number one, and never on even-numbered autozooids. This pattern of occurrence obtained in all the internodes, except the first, in all the main 'stem sequences', in all the colonies.

5.2.2.2.4 Lateral avicularia

Lateral avicularia occurred distolaterally on a number of autozooids. Although generally small, much larger forms occurred intermittently, usually on one of the sub-apical autozooids.

Was their level of occurrence similar in the two populations? Was the occurrence of lateral avicularia related to autozooid number?

Autozooid number	Musselwick, Pembrokeshire			Bay Fine, Isle of Man		
	Lateral avicularia		Percentage occurrence	Lateral avicularia		Percentage occurrence
	Present	Absent		Present	Absent	
4	7	34	17	46	5	90
3	14	26	35	45	4	92
2	1	40	2	36	12	75
1	6	35	15	45	5	90
Total	28	135	17	172	26	87

Chi-Square, Yates' Correction for Continuity 172.924 P= < 0.001 (By site)
Chi-Square 11.357 P= < 0.001 (By autozooid number)

Table 5.3 Lateral avicularia presence/absence by autozooid number, in the two populations (apical autozooids and 'x' and 'i' cases excluded)

Table 5.3 shows that lateral avicularia occurred five times more frequently on the Isle of Man material. Here they occurred almost equally on the variously numbered autozooids. On the Musselwick material they were virtually absent from autozooid No. 2, and occurred much more frequently on autozooid No. 3, than autozooid Nos. 1 and 4.

5.2.2.2.5 Vibracula

Was their level of occurrence similar in the two populations? Was the occurrence of vibracula related to autozooid number?

Autozooid number	Musselwick, Pembrokeshire			Bay Fine, Isle of Man		
	Vibracula		Percentage occurrence	Vibracula		Percentage occurrence
	Present	Absent		Present	Absent	
4	43	4	91	47	4	92
3	10	34	23	11	36	23
1	35	8	81	47	4	92
Total	88	46	66	105	44	70

Chi-Square, Yates' Correction for Continuity .544 P= 0.461 (By site)
Chi-square 126.377 P= < 0.001 (By autozooid number)

Table 5.4 Vibracula presence/absence by autozooid number, in the two populations (apical and No. 2 autozooids and 'x' and 'i' cases excluded)

Table 5.4 shows no evidence of variation between populations in respect of vibracula, in either the overall level, ~68%, or pattern of occurrence. Although the numbers are small, it is clear that vibracula occurrence was strongly related to autozooid number. Vibracula occurred ~4 times more frequently on autozooids Nos. 1 and 4, than on No. 3.

As discussed in Materials and Methods, Section 4.5.1, a single axial vibraculum was always produced by the No. 2 two autozooid of the 'stem' internode, following a bifurcation. The pattern of presence/absence of these vibracula was absolutely constant, and their actual siting was in the axil: assigning them to one internode or the other would have been meaningless. As a result, and because I was undecided if the 'absences' should be treated as such or 'not possible', I treated them simply as 'axilliary' and excluded them from the general analysis. They occurred on all No. 2 autozooids in 'stems' and were absent from these autozooids in 'branches'.

5.2.2.2.6 Rhizoids

The colony was attached to the substratum by tubular rhizoids, which terminated in holdfast like attachment discs. Rhizoids developed from all of the first few autozooids, but soon occurred less frequently and were absent from the more distal internodes.

5.2.2.3 OVICELLS

No ovicells were present in any of the 30 main 'stem sequences'.

5.2.2.4 INTER-COLONY VARIATION

There was no evidence of inter-colony variation except in the case of lateral avicularia, the level of occurrence of which did vary between colonies of the Musselwick population.

5.2.2.5 INTER-POPULATION VARIATION

There was no evidence of inter-population variation with the notable exception of the substantial variation in lateral avicularia occurrence.

All of the above descriptions were based on zooids in the main 'stem sequences' of colonies. It was apparent, although not quantified, that the internode composition of these main 'stem sequences' was very different from that of the colonies overall. Colonies were not homogeneous in respect of internode lengths, and immediately demonstrated that one cannot extrapolate the findings in respect of main 'stem sequences' to complete colonies.

5.3 DETAILED INVESTIGATION INTO THE SPATIAL ARRANGEMENT OF AUTOZOOIDS AND HETEROZOOIDS, WITHIN A SINGLE COLONY FROM MUSSELWICK

5.3.1 Introduction

The preliminary study of 30 'main stem sequences' had not revealed any obvious differences between colonies, or populations, except in respect of the level of occurrence of lateral avicularia. For that polymorph there was variation within the Musselwick population, and between the two populations. The study showed that main 'stem sequences' were, in respect of the lengths of their constituent internodes, very different from the colony generally. This demonstrated that there was some cryptic organization of various length internodes within the colony. It was therefore decided to look in detail at a single colony.

A general indication of the uneven nature of colony growth can be seen from the following table, which shows estimates of the relative sizes of the 'segments' of the colony, which developed from 5th generation internodes E1 to E8; and the actual autozooid numbers in all the internodes which generated from internodes E9 to E16. (The estimates were made prior to the actual investigation).

First internode of 'segment'	Estimated or actual size of 'segment'
E 1	Medium
E 2	Large
E 3	Very large
E 4	Small
E 5	Very large
E 6	Small
E 7	Small
E 8	Very large
E 9	91 autozooids
E10	2628 autozooids
E11	2549 autozooids
E12	455 autozooids
E13	125 autozooids
E14	2496 autozooids
E15	654 autozooids
E16	518 autozooids

Table 5.5 The estimated and actual sizes of the 16 colony 'segments' which developed from the fifth generation of internodes

The subjective evaluations of the 'segments' developing from E1 to E8 were necessarily approximate, but they were very similar in extent to the actual figures of E9 to E16 in Table 5.5, in that generally sections were either 'small' (91-654) or 'very large' (2496-2628).

As discussed in Chapter 4, Materials and Methods, Section 4.5.3.1, I felt that if I divided the colony down an imaginary central axis, and recorded information in relation to one half, the scale and nature of any lateral variation would become apparent. The data in this study, except for that relating to the 10 longest 'stem sequences' which were in relation to the complete colony, relate to one half of the colony.

The astogenetic pattern of *S. reptans,* and indeed many other cellularine bryozoans, involves the repeated bifurcation of internodes, and therefore the number of internodes potentially doubles in each new generation. Clearly this is an aspect of the ability of such colonies to grow very quickly, but how important an aspect? It seemed reasonable to me, prior to this study, that growth would initially be exponential, and then, as growth proceeded the rate of increase would reduce at a progressively increasing rate but I could not quantify this, even approximately.

5.3.2 Results

The raw data are in Appendix 'D'

5.3.2.1 AUTOZOOIDS, INTERNODES AND 'STEM SEQUENCES'

5.3.2.1.1 The interrelationship of internodes at bifurcations

Although, as discussed in Section 4.3, it is possible to describe a colony as if it were two dimensional, it obviously is not. The three dimensional nature of a colony derives primarily from the angulation of internodes in relation to one another at bifurcations. Firstly, the angles between the frontal surface of a parent internode and the two produced at its bifurcation were less than 180°, especially so in respect of its 'branch' internode. Secondly, this internode was also inturned, so that its frontal face, relative to that of its companion 'stem' internode, was much less than 180°. In addition, the internodes themselves, most noticeably the longer ones, were slightly concave, lengthwise. As a result of all of the above, colonies are essentially cup-shaped externally, although not empty internally.

The branching pattern, as described by Lutaud (1953) and detailed in Figure 5.3, is absolutely constant in respect of the way in which 'stem' and 'branch' internodes are produced at bifurcations. As a result of this, if one ignores the first two generations of internodes, in which there are only one and two internodes respectively, the arrangement of 'stem' and 'branch' internodes is the same within all generations of internodes. If all of the internodes within a generation (including any which are missing) are numbered, internode number one is a 'branch', numbers two and three are 'stems', number four is a 'branch', and the pattern is repeated across the generation.

The constancy of the branching pattern is in marked contrast to the resulting arrangement of internodes within a colony.

5.3.2.1.2 The numbers and lengths of internodes, complete and incomplete, and in relation to internode generation

How did the numbers of internodes, complete and incomplete, vary with internode generation?

Internode generation	Complete		Incomplete		Total	
	Number	Percentage occurrence	Number	Percentage occurrence	Number	Percentage occurrence
28			1	0.1	1	0.1
27	1	0.1	3	0.4	4	0.2
26	2	0.2	4	0.6	6	0.3
25	3	0.3	19	2.7	22	1.3
24	16	1.6	19	2.7	35	2.0
23	21	2.0	27	3.8	48	2.8
22	29	2.8	50	7.1	79	4.6
21	50	4.9	67	9.5	117	6.8
20	70	6.8	42	6.0	112	6.5
19	84	8.2	63	8.9	147	8.5
18	84	8.2	82	11.6	166	9.6
17	94	9.2	73	10.4	167	9.7
16	93	9.1	50	7.1	143	8.3
15	81	7.9	24	3.4	105	6.1
14	59	5.8	33	4.7	92	5.3
13	61	5.9	26	3.7	87	5.0
12	48	4.7	30	4.3	78	4.5
11	45	4.4	33	4.7	78	4.5
10	46	4.5	21	3.0	67	3.9
9	42	4.1	20	2.8	62	3.6
8	38	3.7	13	1.8	51	2.9
7	27	2.6	4	0.6	31	1.8
6	16	1.6			16	0.9
5	8	0.8			8	0.5
4	4	0.4			4	0.2
3	2	0.2			2	0.1
2	1	0.1			1	0.1
1	1	0.1			1	0.1
Total	1026	100.0	704	100.0	1730	100.0

Table 5.6 The number and percentage occurrence of complete and incomplete Internodes, by internode 'generation'

Table 5.6 shows that exponential growth, in terms of the number of internodes, ceased after sixth generation of bifurcations. Internode numbers per generation, increased slowly over the succeeding 9 or 10 generations, and then declined rapidly. The number of incomplete internodes, as a proportion, increased generation on

generation, and in distal generations they were more numerous than complete internodes.

For complete internodes was there a relationship between internode length and internode generation?

Internode generation	Number of autozooids in internode							Number of internodes	Mean number of autozooids
	3	4	5	7	9	11	13		
	Frequency								
27			1					1	5.0
26			2					2	5.0
25			3					3	5.0
24			13	3				16	5.4
23			12	9				21	5.9
22			13	15	1			29	6.2
21			21	25	4			50	6.3
20			21	43	6			70	6.6
19			22	57	4	1		84	6.6
18			27	50	6	1		84	6.5
17			33	58	2	1		94	6.4
16			28	58	6	1		93	6.6
15			26	46	8	1		81	6.6
14			18	36	4	1		59	6.6
13			18	34	7	1	1	61	6.8
12			16	29	3			48	6.5
11			17	25	3			45	6.4
10			22	20	4			46	6.2
9			19	17	4	2		42	6.5
8			18	16	4			38	6.3
7			12	15				27	6.1
6			9	7				16	5.9
5			8					8	5.0
4			4					4	5.0
3			2					2	5.0
2	1							1	3.0
1		1						1	4.0
Total	1	1	385	563	66	9	1	1026	6.4

Table 5.7 Lengths of complete internodes by internode generation

Table 5.7 shows a very clear but complex relationship, between the length of (complete) internodes and internode generation. Internodes of five autozooids predominated in the more proximal generations, but as growth proceeded, internodes of seven became the most numerous, and some longer internodes were present. In the more distal regions of the colony this trend was reversed, with internodes of five

again becoming the most abundant. In effect, the longer the internode, the more distal, in terms of internode generation, its first appearance, and the more proximal, its disappearance. The average number of autozooids per internode essentially increased in the more proximal, and decreased in the more distal, generations, and remained high throughout the central generations.

Which internode lengths occurred and how frequently?

Autozooids in internode	Complete		Incomplete	
	Number	Percentage occurrence	Number	Percentage occurrence
1			65	9.2
2			133	18.9
3	1	0.1	86	12.2
4	1	0.1	144	20.5
5	385	37.5	86	12.2
6			87	12.4
7	563	54.9	56	8.0
8			19	2.7
9	66	6.4	13	1.8
10			10	1.4
11	9	0.9	2	0.3
12			2	0.3
13	1	0.1	1	0.1
Total	1026	100.0	704	100.0

Table 5.8 The number and percentage occurrence of the various length internodes, both complete and incomplete

From Table 5.8 it was apparent:-

- All complete internodes (except the first) were of an odd number of autozooids.
- Complete internode lengths ranged from 3 to 13 autozooids.
- Internodes of seven autozooids were the most numerous, and together with those of five constituted > 90% of complete internodes.
- 40% of internodes were incomplete.

(Incomplete internodes occurred within all but the first six generations of internodes, and it is unfortunate that one cannot determine whether these were still growing or had ceased to grow, when the colony was collected. The stage of development of the

scuta on the most distal autozooids of an internode enabled unbroken and broken internodes to be differentiated, but does not throw any light on whether or not the former were still growing when the colony was collected).

5.3.2.1.3 'Stem' and 'branch' internodes

Were there equal numbers of 'stems' and 'branches', and was there a relationship between the length of a complete internode, and whether it was a 'stem' or a 'branch'?

Autozooids in internode	'Stem' internodes		'Branch' internodes	
	Frequency	Percentage occurrence	Frequency	Percentage occurrence
3			1	100.0
4	1	100.0		
5	204	53.0	181	47.0
7	299	53.1	264	46.9
9	43	65.2	23	34.8
11	7	77.8	2	22.2
13	1	100.0		
Total	555	54.1	471	45.9

Chi-Square 6.877 P= 0 .009 ('stems' and 'branches')
Chi-Square 8.568 P= 0.199 ('stems' and 'branches' by length.)

Table 5.9 The frequency and percentage occurrence of complete 'stem' and 'branch' internodes, by length

Two features are apparent from Table 5.9:-

- For complete internodes there were ~17.5% more 'stems' than 'branches'.
- The longer an internode the more likely it was to be a 'stem'.

The length of incomplete internodes was of little value but were there equal numbers of 'stems' and 'branches'?

Internode type	Frequency	Percentage occurrence
'stem'	386	54.8
'branch'	318	45.2

Chi-Square 6.568 P= 0.01

Table 5.10 The frequency and percentage occurrence of incomplete 'stem' and 'branch' internodes

Table 5.10 shows that there were ~21% more 'stem' than 'branch' internodes.

How did the mean lengths of complete and incomplete 'stem' and 'branch' internodes compare?

Internode characteristics	Number of autozooids	Number of internodes	Mean number of autozooids
Complete 'stems'	3594	555	6.48
Complete 'branches'	2985	471	6.34
Incomplete 'stems'	1606	386	4.16
Incomplete 'branches'	1331	318	4.19

Table 5.11 The mean number of autozooids in complete and incomplete internodes, in 'stem' and 'branch' internodes

Table 5.11 shows that, on average, incomplete internodes were >2 autozooids shorter than complete internodes. (This apparently trivial result is included because the situation in respect of *T. inopinata,* detailed in Chapter 6, was very different).

5.3.2.1.4 'Stem sequences'

The differentiation of 'stem' and 'branch' internodes, led to the concept of 'stem sequences'. 'Stem sequences' are sequences of internodes, all of which deviate from their predecessor by the smaller of the two angles possible. All 'stem sequences', except the one which originates with the very first internode of the colony, commence with a 'branch', and all subsequent internodes are 'stems'.

5.3.2.1.4.1 The lengths of 'stem sequences'

How frequently did 'stem sequences' of different lengths occur, and how did their occurrence relate to their generation of origin?

Number of internodes in 'stem sequence'	Internode generation of origin													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Frequency													
26			1											
25														
24														
23														
22				1										
21														
20														
19														
18		1												
17														
16														
15														
14														
13									1	2				
12											1			
11										1				
10					1									
9										1	1			1
8				1	1					1	1		1	
7											1		1	1
6							2		2			1		2
5						2	2	2				2		3
4					1	3	1	1	4	2	3	1	4	4
3					1	2	4	4	5	2	4	5	7	8
2						1	3	7	5	8	7	12	12	4
1							3	11	11	13	18	14	16	16
Total	0	1	1	2	4	8	15	25	28	30	36	35	41	39

Table 5.12 The occurrence, in terms of 'stem sequence' lengths (number of all internodes) of all of the 'stem sequences', in relation to their generation of origin

(This Table continues, for 'stem sequences' originating in the subsequent 13 generations of internodes on the following page).

Number of internodes in 'stem sequence'	Internode generation of origin													Total
	15	16	17	18	19	20	21	22	23	24	25	26	27	
	Frequency													
26														1
25														
24														
23														
22														1
21														
20														
19														
18														1
17														
16														
15														
14														
13														3
12														1
11														1
10														1
9														3
8	1			2										8
7	1													4
6	1	1		1										10
5	5		2		2			1						21
4	2	3	2	2	4	2	1	2						42
3	11	16	9	7	9	7	5	2	3					111
2	15	24	28	27	16	22	12	7	7	6	1	1		225
1	16	22	34	37	38	20	36	24	11	9	5	2	2	358
Total	52	66	75	76	69	51	54	36	21	15	6	3	2	791

Table 5.12 (cont.) The occurrence, in terms of 'stem sequence' lengths (number of all internodes) of all of the 'stem sequences', in relation to their generation of origin

Table 5.12 shows that there were a small number of long 'stem sequences', all of which originated in the first six generations of internodes, and a great number of short 'stem sequences', which originated beyond the first three generations. One cannot, therefore, distinguish a hierarchy of orders of 'stem sequences', related simply to their generation of origin.

5.3.2.1.4.2 The relationship between the length of complete internodes and their position within a 'stem sequence'

Was there a relationship between the length of a complete internode, and its position within a 'stem sequence'?

(The data relating to the lengths of all complete internodes, and their sequence within the 'stem sequences' initiated in the first 13 generations of internodes, is in Appendix 'E').

Numerical position within'stem sequence'	Internode length					
	3	5	7	9	11	13
25		1				
24		1				
23		1				
22		1				
21		1	1			
20		2				
19		2				
18		2	1			
17		3				
16		3				
15		3				
14		3				
13		3				
12		5	1			
11		6	1			
10		6	2			
9		9				
8		11	2			
7		13	7			
6		17	6	1		
5		19	17	1		
4		26	29	1		
3		41	67	2	1	
2		25	165	38	6	1
1	1	181	264	23	2	
Total	1	385	563	66	9	1

Table 5.13 Lengths of complete internodes by their numerical position within their 'stem sequence'

Table 5.13 shows that internodes of seven or more autozooids were essentially restricted to the proximal positioned internodes, within a 'stem sequence'.

5.3.2.1.4.3 Internode lengths and their sequence within the ten longest 'stem sequences'

Did these long 'stem sequences' exhibit any particular characteristics, in terms of the lengths and/or sequence, of their constituent internodes?

Internode generation	Internode lengths and sequence									
27				5						
26				5						
25			5	5						
24			5	5	5	5			5	
23			5	7	5	5	5		5	
22			5	5	5	5	5		5	
21			5	5	5	5	5		5	
20			5	5	5	5	5	5	5	5
19		7	5	5	5	5	5	5	5	5
18		5	5	5	5	5	5	5	5	7
17	5	5	5	5	5	7	5	5	5	5
16	5	5	5	5	5	5	5	5	5	5
15	7	5	5	5	5	5	5	5	5	5
14	7	5	5	5	5	5	5	5	5	7
13	5	5	5	5	5	5	5	5	5	5
12	5	5	5	5	5	5	5	5	5	5
11	5	5	5	5	5	5	5	5	7	5
10	7	5	5	5	5	5	5	5	5	5
9	5	5	5	5	5	5	5	5	5	5
8	7	5	5	5	5	5	5	5	5	5
7	7	5	5	5	5	5	5	5	7	7
6	5	5	5	5	5	5	5	5	5	5
5	5	5	5	5	5	5	5	5		
4	5	5	5	5	5					
3	5	5	5	5						
2	3	3								
1	4									
Internode of origin	A1	B2	C1	C4	D5	E1	E5	E8	F4	F8

Table 5.14 The internode lengths and their sequence in the 10 longest 'stem sequences'

Table 5.14 shows that that the 10 longest 'stem sequences', of the complete colony, had an identical internode composition to that of the main 'stem sequences' of the preliminary study. Four 'stem sequences' consisted entirely of internodes of five autozooids and a further three had only one internode of seven autozooids. The main

'stem sequence', in the first column, is the one which deviated most from the general pattern, containing no fewer than five internodes of seven. No internodes of more than seven autozooids were present in any of the long 'stem sequences'.

It is not only main 'stem sequences' which are constituted overwhelmingly of internodes of five autozooids. All of the 10 longest 'stem sequences' within this colony had this internode constitution. The above has to be seen against a background of the colony as a whole, in which internodes of seven outnumbered those of five autozooids, by virtually three to two, and internodes of more than seven autozooids constituted ~7% of the total.

The table shows no obvious sequences of internodes, but it is noticeable that the only internode of four autozooids was in the first, and that the two internodes of three autozooids were both in the second generation.

How did the internode composition of the colony compare with that of the 10 longest 'stem sequences'?

Autozooids in Internode	Colony		10 longest 'stem sequences'	
	Number	Percentage occurrence	Number	Percentage occurrence
3	1	0.1	2	1.1
4	1	0.1	1	0.5
5	385	37.5	177	91.7
7	563	54.9	13	6.7
9	66	6.4		
11	9	0.9		
13	1	0.1		
Total	1026	100.0	193	100.0

Chi-Square 267.779 P= < 0.001

Table 5.15 The frequency and percentage occurrence of complete internodes of different numbers of autozooids, in the colony, and in the 10 longest 'stem sequences'

Table 5.15 shows that the difference in internode composition between the long 'stem sequences' and the colonies as a whole, apparent, but not quantified in the original investigation, extended here into the small number of very long 'stem sequences' present in this colony. In this colony 55% of complete internodes were of

seven autozooids, whilst in the 10 longest 'stem sequences' they constituted only 7%. Whilst within the 10 longest 'stem sequences' over 90% of internodes were of five autozooids, within the colony they constituted 37.5%. Within the colony, some 7% of internodes were of nine or more autozooids, but nonesuch were present within the 10 longest 'stem sequences'.

To establish, whether or not the internode composition of long 'stem sequences' was a primary feature, or secondary, resulting from other characteristics, two differences between the colony and its long 'stem sequences' had to be investigated.

- 1) The proportions of the different length internodes in the various generations of internodes were very different in the colony and the long 'stem sequences'. In order to establish whether or not this difference, in itself, could account for the observed difference in internode length composition, it was only necessary to calculate the numbers of the various lengths of complete internodes, that one would expect to find in the 10 longest 'stem sequences', if the proportions of these, within the various internode generations, were the same as in the colony.

Did differences in the proportions of the different length internodes per generation, within the colony, and within the 10 longest 'stem sequences', account for the differences in the internode composition of the two?

Inter-node gener-ation.	Number of autozooids in internode													
	3		4		5		7		9		11		13	
	Frequency													
	Act	Exp	Act	Exp	Act	Exp	Act	Exp	Act	Exp	Act	Exp	Act	Ex
27					1	1.0								
26					1	1.0								
25					2	2.0								
24					5	4.1	0	0.9						
23					5	3.6	1	2.4						
22					6	2.7	0	3.1	0	0.2				
21					6	2.4	0	3.1	0	0.5				
20					8	2.3	0	5.0	0	0.7				
19					8	2.3	1	6.3	0	0.3	0	0.1		
18					8	2.9	1	5.3	0	0.6	0	0.1		
17					9	3.5	1	6.2	0	0.2	0	0.1		
16					10	3.0	0	6.2	0	0.6	0	0.2		
15					9	3.2	1	5.7	0	1.0	0	0.1		
14					8	3.1	2	6.0	0	0.7	0	0.2		
13					10	2.9	0	5.6	0	1.1	0	0.2	0	0.2
12					10	3.3	0	6.0	0	0.6				
11					9	3.8	1	5.5	0	0.7				
10					9	4.8	1	4.3	0	0.9				
9					10	4.5	0	4.0	0	1.0	0	0.5		
8					9	4.7	1	4.2	0	1.1				
7					7	4.4	3	5.6						
6					10	5.6	0	4.4						
5					8	8.0								
4					5	5.0								
3					4	4.0								
2	2	2.0												
1			1	1.0										
Total	2	2.0	1	1.0	177	88.1	13	89.8	0	10.2	0	1.5	0	0.2

Table 5.16 Comparison of the actual numbers of the various lengths of complete internodes in the 10 longest 'stem sequences', with the numbers expected if the proportions of the different internode lengths in the various internode generations, were the same as in the colony

Table 5.16 shows that if the proportions of the various internode lengths in the 10 longest 'stem sequences' were the same as in the colony, one would have expected

88 internodes of five, 90 of seven autozooids, and ~12 of internodes of more than seven autozooids.

If the figures in Table 5.16 are added to those in Table 5.15, did they account for the differences within that table?

Autozooids in internode	Colony		10 longest 'stem sequences'			
			Actual internode composition		Expected internode composition	
	Number	%	Number	%	Number	%
3	1	0.1	2	1.1	2	1.1
4	1	0.1	1	0.5	1	0.5
5	385	37.5	177	91.7	88	45.6
7	563	54.9	13	6.7	90	46.6
9	66	6.4			11	5.7
11	9	0.9			1	0.5
13	1	0.1				
Total	1026	100.0	193	100.0	193	100.0

Chi-Square 168.89 P= < 0.001 (Actual and expected)

Table 5.17 The figures derived from Table 5.16 are added to the original comparison between the internode length composition of the colony and the 10 longest 'stem sequences' (Table 5.15)

Table 5.17 shows that this factor, the difference between the proportions of the different internode generations within the colony and within the 10 longest 'stem sequences', could account for little of the observed variation. In the 10 longest 'stem sequences' over 90% of internodes were of five, some 7% of seven autozooids; and no internodes of more than seven autozooids were present. The 'expected' figures were ~46% internodes of five, ~47% internodes of seven and ~6% of internodes with more than seven autozooids.

- 2) The constituent internodes of the ten longest 'stem sequences' differed from those of the colony in that the former, by definition, consisted virtually entirely of 'stem' internodes. One would have expected the colony to consist of approximately equal numbers of each. (In this colony there were actually ~15% more 'stem' than 'branch', complete internodes).

The percentages of 'stem' and 'branch' internodes of five and seven autozooids were almost identical to the percentage obtaining overall (see Table 5.9) and there was no imbalance here to which the observed differences could be ascribed.

The internode composition of the long 'stem sequences', does not result from other characteristics of the spatial arrangement of internodes, it is a primary feature.

How did the mean number of autozooids per internode compare in the colony and in the 10 longest 'stem sequences'?

	Number of autozooids	Number of internodes	Mean number of autozooids
Colony	6579	1026	6.41
10 longest 'stem sequences'	986	193	5.11

Chi-Square 50.93 P= < 0.001

Table 5.18 The mean length of complete internodes in the colony, and in the 10 longest 'stem sequences'

Table 5.18 shows that, on average, internodes of long 'stem sequences' had 1.3 fewer autozooids than those within the colony.

5.3.2.1.4.4 The spatial arrangement of internodes within the colony, particularly in relation to the long 'stem sequences'

An indication of the distribution of the 10 longest 'stem sequences' in the colony can be obtained by looking at the distribution of their internodes in the 16th generation, generation 'P'. Theoretically some 32,000+ internodes were possible within this generation. The actual internode numbers, indicating the lateral position of the internodes of the 10 longest 'stem sequences' within this generation, are:-

1,366; 3,714; 5,462; 7,510; 9,558; 10,923; 15,019; 19,115; 21,846; 27,307.

They were well, if not evenly, spaced.

In the proximal region of the colony all possible internodes did develop, but as growth proceeded there was a 'thinning out', which increased in magnitude in the

more distal regions. The manner in which internodes of these were distributed laterally, can be seen in the details below:-

In the 18th generation, 'R', ~130,000 internodes were theoretically possible. In the half of the colony which was 'mapped', some 166 internodes were present, occurring within the numerical range of ~ 65,000-130,000. (It was the right-hand half of the colony which was mapped).

The clumped nature of their distribution is evidenced by the following:-

* 52 internodes occurred between 76,373 and 76,720
* 65 internodes occurred between 87,201 and 87,808
* 43 internodes occurred between 109,138 and 109,400
  6 internodes occurred between 119,977 and 119,983 (all were incomplete)

(No other internodes were produced within this half of the colony).

* Each of these three large clusters had an internode of one of the ten longest 'stem sequences' within it.

The spatial arrangement of internodes in relation to the long 'stem sequences' is shown in Figure 5.4. It shows, for certain generations, for the half of the colony which was 'mapped', the proportion of available internode positions actually 'occupied', and how these were distributed in these generations.

- The dashed vertical lines indicate the long 'stem sequences'.
- The dotted vertical line a 'stem sequence' which was not very long but which had the internode composition of such a 'stem sequence' (possibly damaged).
- The boxes and vertical lines within each generation the lateral extent of internode occurrence.

The figure inevitably gives a very false impression of the numbers of internodes occurring in each generation. 90% of all internodes occurred distal to generation 'I', and 60% distal to generation 'O'.

Figure 5.4 The positions of internodes produced, relative to potential but unoccupied 'sites', in relation to long 'stem sequences', in certain internode generations within one half of the colony (Musselwick).

Figure 5.4 shows, for the half of the colony which was mapped, the relationship between the long 'stem sequences' and the distribution of internodes within a number of internode generations. Initially internodes are produced in all positions open to them but as growth continues, gaps between groups of internodes appear which rapidly increase in width. The concentration of narrow groups of internodes around long 'stem sequences' in the central and distal regions of the colony is very pronounced. It is also apparent from Figure 5.4, that even when a long 'stem sequence' came to an end, growth often continued in that direction. The long 'stem sequences originating with internodes B2 and D8 had growth continuing in the direction of those 'stem sequences', for four and seven generations respectively, beyond the extent of the long 'stem sequences' themselves.

Figure 5.4 does not show the actual spatial arrangement of internodes within the 'aggregations' of internodes associated with long 'stem sequences'. Although the number of internodes in each generation of internodes is theoretically double the number of its predecessor, scaling requires each generation to be represented by an identical space. The figure also does not provide any information on the distribution of the different lengths of internode.

The problem can be largely circumvented, and a more realistic picture obtained, in these respects, if a laterally, and vertically, limited region of the colony, in relation to one long 'stem sequence', is considered, allowing an identical scale to be used for each generation.

Figure 5.5, shows the lengths, whether they were complete or incomplete, and their actual position within their generation, of the vast majority of the internodes which were generated, from generations 'J' to 'Z', from a long 'stem sequence' which developed from internode 'D5'.

- All internodes are drawn to scale, one square = one autozooid.
- Incomplete internodes are indicated by a dot above the most distal autozooid.
- Complete internodes of five autozooids are shaded.
- The horizontal line beneath each generation of internodes, indicates the extent of their possible occurrence, and the X / Y figures at the sides, the number of internodes which occurred, and the number of internodes possible, beyond the margins of the figure.

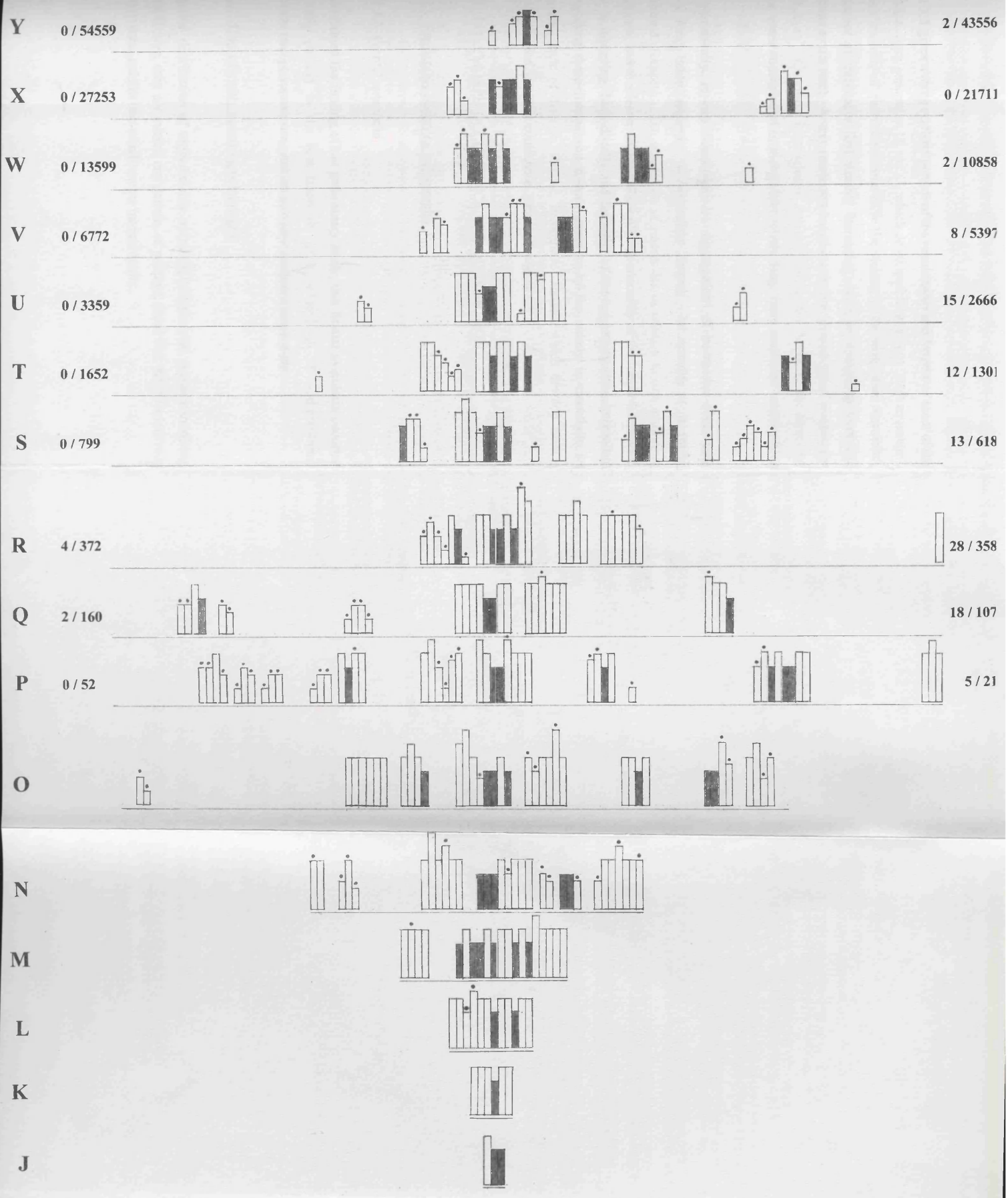


Figure 5.5 The pattern of internode presence and absence (and their length) for all of the internodes which could have developed from the long 'stem sequence' which developed from internode 'D5' (Musselwick).

Figure 5.5 gives a more realistic picture of the relationship and lengths of most of the internodes, from generation 'J' to 'Z', which developed from a long 'stem sequence'. The vast majority of internodes occurred in the vicinity of the long 'stem sequence', the number of internodes first steadily increasing and then decreasing from one generation to the next. A very small number of internodes formed 'arms' outside the central 'core'. Complete internodes of five autozooids, the shorter of the abundant lengths, were concentrated in the vicinity of the long 'stem sequence' and in these 'arms'.

Diagrammatically, in two dimensions, an 'aggregation' of internodes developing from a long 'stem sequence' is lanceolate shaped. In actuality it is three-dimensional, fusiform. The angulation of internodes in relation to one another at bifurcations, and to a lesser degree the lengthwise concavity of the longer internodes, results in incurving, both distally and laterally, of the 'aggregation' of internodes. The resulting three-dimensional overall structure of the colony is essentially an incomplete circle of discrete, slender, incomplete 'flasks', which develop from a vertically limited, laterally continuous, proximal region.

5.3.2.2 POLYMORPH OCCURRENCE AND DISTRIBUTION

5.3.2.2.1 Predictably occurring polymorphs

5.3.2.2.1.1 Frontal avicularia

This confirmed the finding of the preliminary study, that frontal avicularia occurred predictably (except in the first internode of a colony) on all odd-numbered autozooids except number one, and on no even-numbered autozooids.

5.3.2.2.2 Unpredictably occurring polymorphs

The spatial distribution of unpredictably occurring polymorphs, perhaps involving external factors, may not exhibit any pattern, or perhaps, given that these factors may vary over time, exhibit one which occurs intermittently.

For polymorphs of unpredictable occurrence (lateral avicularia, vibracula and rhizoids) it was necessary to estimate their distribution by sampling the colony. The problem was to establish a region which constituted a genuinely representative sample of the whole. The limited vertical dimension was not a problem, and the discovery of the existence of largely discrete 'aggregations' of internodes, each centred on a long 'stem sequence', suggested that these could provide the necessary lateral element previously lacking.

Two separate 'aggregations' of internodes, each in association with their long 'stem sequence', were investigated. In the first, selected generations of internodes of a long 'stem sequence', together with all of the internodes which developed, in those generations, from one side of it, were recorded. This will be referred to as Aggregation 'A'. In the second, a shorter long 'stem sequence', together with all of the internodes which developed from it, were recorded. This will be referred to as Aggregation 'B'.

(The distribution of all three polymorphs in Aggregation 'A' is shown in Figure 5.6).

Figure 5.6 shows, for Aggregation 'A', the distribution of lateral avicularia, vibracula and rhizoids in relation to autozooids and internodes, within certain generations of a long 'stem sequence', and all of the internodes within these, which developed from one side of it.

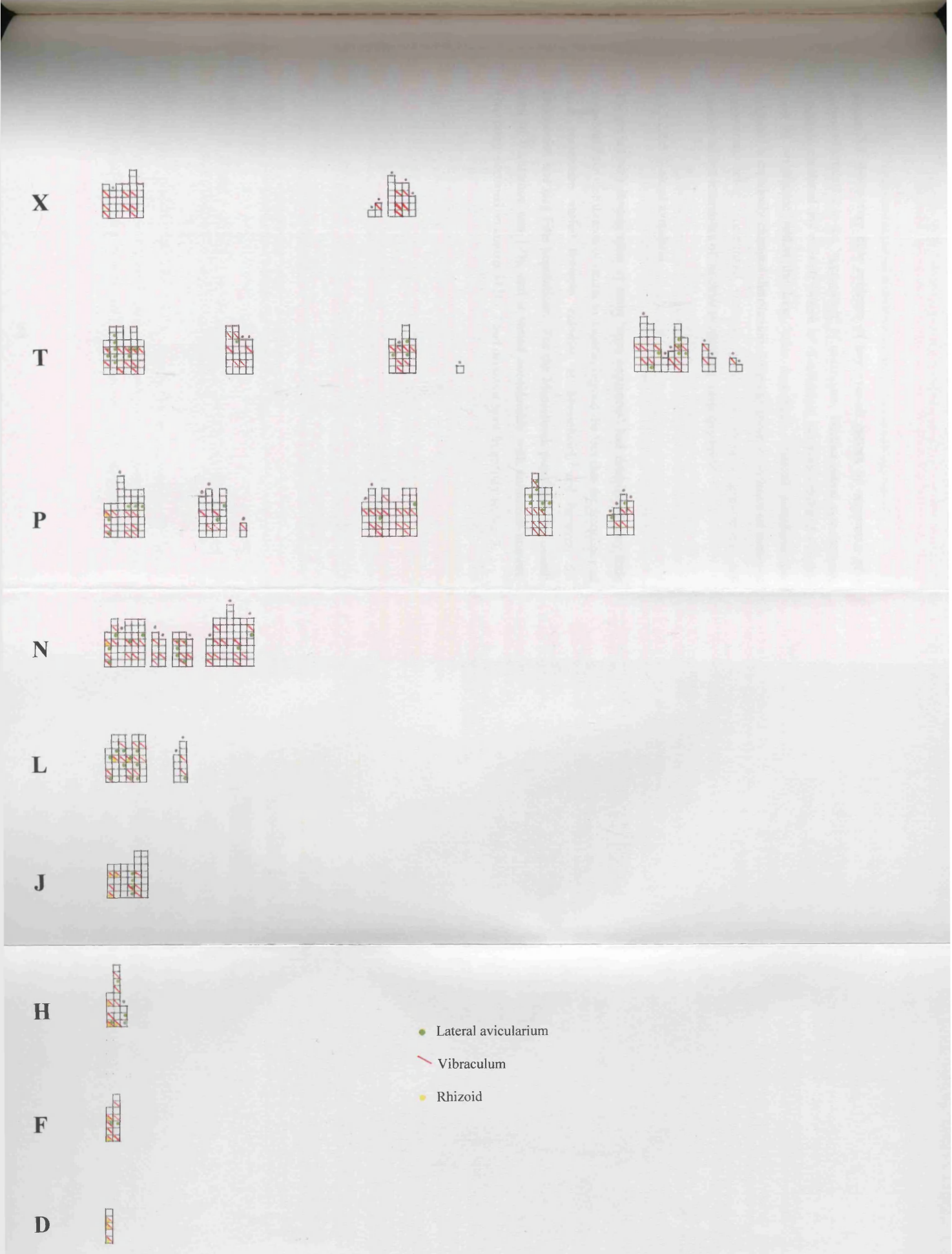


Figure 5.6 The spatial distribution of lateral avicularia, vibracula and rhizoids in relation to the internodes which developed in certain generations, from one side of the long 'stem sequence' which developed from internode 'D5'; Aggregation 'A' (Musselwick).

Figure 5.6 shows very little evidence of any over-all pattern of occurrence of a polymorph, relative to the 'aggregation' of internodes. Neither lateral avicularia nor vibracula exhibited any over-all pattern of distribution, but both exhibited a higher level of occurrence within the long 'stem sequence'. Lateral avicularia also exhibited a decidedly clumped distribution. Rhizoids showed evidence of zones of occurrence, being concentrated vertically within the proximal generations, and laterally within internodes of, or close to, the long 'stem sequence'.

5.3.2.2.2.1 Lateral avicularia

The preliminary investigation of main 'stem sequences' had established very little regarding their distribution of lateral avicularia, beyond the fact that the frequency of their occurrence varied between colonies at Musselwick and between the Musselwick and Bay Fine populations. For the Musselwick population the overall level of occurrence was 17%, and it varied considerably with autozooid number. They rarely occurred on autozooid No. 2 and occurred most frequently on No. 3.

Was lateral avicularia presence or absence related to internode generation?

Aggregation 'A'			
Internode generation	Lateral avicularia		Percentage occurrence
	Present	Absent	
24	0	36	0
20	20	78	20
16	18	118	13
14	12	68	15
12	11	29	27
10	4	18	18
8	4	11	27
6	2	8	20
Total	71	366	16

Aggregation 'B'			
Internode generation	Lateral avicularia		Percentage occurrence
	Present	Absent	
18	0	10	0
17	1	24	4
16	2	16	11
15	2	20	9
14	3	35	8
13	9	77	10
12	6	43	12
11	15	45	25
10	22	47	32
9	22	46	32
8	11	25	31
7	4	16	20
6	5	5	50
5	1	0	100
Total	103	409	20

Aggregation 'A'- Chi-Square 14.312 P= 0.046 (3/19)
Aggregation 'B'- Chi-Square 44.679 P= < 0.001 (7/25)

Table 5.19 Lateral avicularia presence or absence by internode generation (apical autozooids and 'x' and 'i' cases excluded)

There is some evidence in Table 5.19 to suggest that in Aggregation 'A' lateral avicularia occurrence was related to internode generation. In Aggregation 'B', there was evidence of a decrease in the level of occurrence, in more distal generations.

Was lateral avicularia presence or absence related to whether an internode was a 'stem' or a 'branch'?

Internode type	Aggregation 'A'			Aggregation 'B'		
	Lateral avicularia		Percentage occurrence	Lateral avicularia		Percentage occurrence
	Present	Absent		Present	Absent	
'stem'	43	187	19	62	226	22
'branch'	28	179	14	41	183	18
Total	71	366	16	103	409	20

Aggregation 'A'- Chi-Square, Yates' Correction for Continuity 1.776 P= 0.183
Aggregation 'B'- Chi-Square, Yates' Correction for Continuity .627 P= 0.429

Table 5.20 Lateral avicularia presence or absence by whether an internode was a 'stem' or a 'branch' (apical autozooids and 'x' and 'i' cases excluded)

Table 5.20 shows that lateral avicularia occurred more frequently in 'stem' than 'branch' internodes, but not to any significant extent.

Was lateral avicularia presence or absence related to whether an internode was complete or incomplete?

Internode type	Aggregation 'A'			Aggregation 'B'		
	Lateral avicularia		Percentage occurrence	Lateral avicularia		Percentage occurrence
	Present	Absent		Present	Absent	
Complete	65	262	20	92	299	24
Incomplete	6	104	5	11	110	9
Total	71	366	16	103	409	20

Aggregation 'A'- Chi-Square, Yates' Correction for Continuity 11.546 P= 0.001
Aggregation 'B'- Chi-Square, Yates' Correction for Continuity 11.106 P= 0.001

Table 5.21 Lateral avicularia presence or absence by whether an internode was complete or incomplete (apical autozooids and 'x' and 'i' cases excluded)

Table 5.21 shows that lateral avicularia occurred ~three to four times more frequently in complete than incomplete internodes.

Was lateral avicularia presence or absence related to autozooid position within an internode?

Autozooid position	Aggregation 'A'			Aggregation 'B'		
	Lateral avicularia		Percentage occurrence	Lateral avicularia		Percentage occurrence
	Present	Absent		Present	Absent	
Sub-apical	41	87	32	47	96	33
Proximal	27	263	9	50	287	15
Total	68	350	16	97	383	20

Aggregation 'A'- Chi-Square, Yates' Correction for Continuity 32.008 P= < 0.001
Aggregation 'B'- Chi-Square, Yates' Correction for Continuity 19.139 P= < 0.001

Table 5.22 Lateral avicularia presence or absence by autozooid position within an internode (apical autozooids and 'x' and 'i' cases excluded)

Table 5.22 shows that lateral avicularia occurred ~2 to 3.5 times more frequently on sub-apical than proximally sited autozooids.

Was lateral avicularia presence or absence related to autozooid number?

Autozooid number	Aggregation 'A'			Aggregation 'B'		
	Lateral avicularia		Percentage occurrence	Lateral avicularia		Percentage occurrence
	Present	Absent		Present	Absent	
10				0	1	0
9				0	1	0
8	1	1	50	1	8	11
7	2	2	50	3	6	33
6	2	36	5	5	45	10
5	19	25	43	19	35	35
4	12	66	15	16	71	18
3	20	66	23	32	59	35
2	4	86	4	3	100	3
1	11	84	12	24	83	22
Total	71	366	16	103	409	20

Aggregation 'A'- Chi-Square 45.733 P= < 0.001 (4/25)
Aggregation 'B'- Chi-Square 45.061 P= < 0.001 (6/30)

Table 5.23 Lateral avicularia presence or absence by autozooid number (apical autozooids and 'x' and 'i' cases excluded)

Table 5.23 confirmed and extended the findings of the preliminary study, and shows that, if one ignores the very small figures for autozooids seven and eight, in

Aggregation 'A'; and nine and ten, in Aggregation 'B'; lateral avicularia occurred more frequently on the odd, than even numbered autozooids of each pair, especially the first.

Was lateral avicularia occurrence related to odd and even-numbered autozooids?

Autozooid number	Aggregation 'A'			Aggregation 'B'		
	Lateral avicularia		Percentage occurrence	Lateral avicularia		Percentage occurrence
	Present	Absent		Present	Absent	
Odd Nos.	52	177	22.7	78	184	29.8
Even Nos.	19	189	9.1	25	225	10.0
Total	71	366	16.2	103	409	20.1

Aggregation 'A'- Chi-Square, Yates' Correction for Continuity 13.776 P= < 0.001
Aggregation 'B'- Chi-Square, Yates' Correction for Continuity 29.900 P= < 0.001

Table 5.24 Lateral avicularia occurrence by odd and even-numbered autozooids (apical autozooids and 'x' and 'i' cases excluded)

Table 5.24 shows that lateral avicularia occurred between two and three times more frequently on odd than even- numbered autozooids.

Within the long 'stem sequences', in Aggregation 'A', 10 lateral avicularia were produced by the 33 autozooids, an occurrence rate of 30%, twice that obtaining in the 'aggregation'. In Aggregation 'B', 12 lateral avicularia were produced by 25 autozooids, an occurrence rate of 48%, more than twice that obtaining in the 'aggregation'.

5.3.2.2.2.2 Vibracula

The initial investigation had established very little regarding the distribution of vibracula, beyond that they occurred very frequently on autozooid Nos. 1 and 4, and much less frequently on autozooid No. 3.

Was vibracula presence or absence related to internode generation?

Aggregation 'A'			
Internode generation	Vibracula		Percentage Occurrence
	Present	Absent	
24	8	28	22
20	23	64	26
16	27	88	23
14	19	54	26
12	11	22	33
10	5	12	29
8	6	6	50
6	6	2	75
4	3	0	100
Total	108	276	28

Aggregation 'B'			
Internode generation	Vibracula		Percentage occurrence
	Present	Absent	
18	0	10	0
17	5	16	24
16	3	13	19
15	3	16	16
14	5	32	14
13	7	65	10
12	11	31	26
11	14	41	25
10	15	46	25
9	21	35	37
8	16	13	55
7	10	6	62
6	5	3	62
5	2	0	100
Total	117	327	26

Aggregation 'A'- Chi-Square 21.790 P= 0.005 (5/28)
Aggregation 'B'- Chi-Square 56.486 P= < 0.001 (6/22)

Table 5.25 Vibracula presence or absence by internode generation (apical and number two autozooids and 'x' and 'i' cases excluded)

Table 5.25 shows, that the level of vibracula occurrence was high in the early, and lower in the later generations. (The numbers for the earliest generations were necessarily small).

Was vibracula presence/absence related to whether an internode was a 'stem' or a 'branch'?

Internode type	Aggregation 'A'			Aggregation 'B'		
	Vibracula		Percentage occurrence	Vibracula		Percentage occurrence
	Present	Absent		Present	Absent	
'stem'	67	134	33	75	183	29
'branch'	41	142	22	42	144	23
Total	108	276	28	117	327	26

Aggregation 'A'- Chi-Square, Yates' Correction for Continuity 5.132 P= 0.023
Aggregation 'B'- Chi-Square, Yates' Correction for Continuity 2.023 P= 0.155

Table 5.26 Vibracula presence or absence by whether an internode was a 'stem' or a 'branch' (apical and number two autozooids and 'x' and 'i' cases excluded)

Table 5.26 shows that vibracula occurred more frequently on autozooids within 'stem' than 'branch' internodes; 50% more frequently in Aggregation 'A', but only 25% in Aggregation 'B'. (But see Table 5.44).

Was vibracula occurrence related to whether an internode was complete or incomplete?

Internode type	Aggregation 'A'			Aggregation 'B'		
	Vibracula		Percentage occurrence	Vibracula		Percentage occurrence
	Present	Absent		Present	Absent	
Complete	92	174	35	110	206	34
Incomplete	16	102	13	7	121	5
Total	108	276	28	117	327	26

Aggregation 'A'- Chi-Square, Yates' Correction for Continuity 16.853 P= < 0.001
Aggregation 'B'- Chi-Square, Yates' Correction for Continuity 38.914 P= < 0.001

Table 5.27 Vibracula presence or absence by whether an internode was complete or incomplete (apical and number two autozooids and 'x' and 'i' cases excluded)

Table 5.27 shows that vibracula occurred much more frequently on autozooids which were in complete as opposed to incomplete internodes, although to a very different degree in the two 'aggregations'. This was not due to their absence from the distal

ends of internodes (incompletely formed distal autozooids, 'i' cases, were ignored); it was frequently due to their complete absence from a number of these internodes.

(Raw data are in Appendix 'F').

Was vibracula occurrence related to autozooid position within an internode?

Autozooid position	Aggregation 'A'			Aggregation 'B'		
	Vibracula		Percentage occurrence	Vibracula		Percentage occurrence
	Present	Absent		Present	Absent	
Sub-apical	38	94	29	44	99	31
Proximal	62	138	31	72	165	30
Total	100	232	30	116	264	30

Aggregation 'A'- Chi-square, Yates' Correction for Continuity .095 P= 0.758
Aggregation 'B'- Chi-Square, Yates' Correction for Continuity .000 P= 1.00

Table 5.28 Vibracula presence or absence by autozooid position within an internode (apical and number two autozooids, and 'x' and 'i' cases excluded)

Table 5.28 gives no indication that vibracula occurrence was related to autozooid position within an internode.

Was vibracula presence/absence related to autozooid number?

Autozooid number	Aggregation 'A'			Aggregation 'B'		
	Vibracula		Percentage occurrence	Vibracula		Percentage occurrence
	Present	Absent		Present	Absent	
10				0	1	0
9				0	1	0
8	2	0	100	0	8	0
7	0	3	0	0	11	0
6	10	29	26	22	33	40
5	0	40	0	1	57	2
4	58	17	77	58	37	61
3	2	81	2	4	97	4
1	28	62	31	32	82	28
Total	100	232	30	117	327	26

Aggregation 'A'- Chi-Square 133.296 P= < 0.001 (4/29)
Aggregation 'B'- Chi-Square 116.129 P= < 0.001 (6/33)

Table 5.29 Vibracula presence or absence by autozooid number (apical and number two autozooids and 'x' and 'i' cases excluded)

Although the numbers are small, Table 5.29 confirms that vibracula occurrence was strongly related to autozooid number. They were virtually absent from all of the odd-numbered autozooids, excluding No. 1, on which they occurred on some ~29% of autozooids. They occurred very frequently on autozooid No. 4, and less frequently on autozooid No. 6.

Within the long 'stem sequence' of Aggregation 'A', 18 vibracula were produced by 26 autozooids, a rate of occurrence of 69%, ~2.5 higher than the level in the 'aggregation'. Within that of Aggregation 'B', 21 vibracula were produced by 36 autozooids, a rate of occurrence of 71%, almost three times higher than the level in the 'aggregation'.

5.3.2.2.2.3 Rhizoids

How does rhizoid presence or absence relate to internode generation?

Aggregation 'A'		
Internode generation	Rhizoid	
	Present	Absent
24		36
20		87
16	1	114
14	1	73
12	2	31
10	3	14
8	3	9
6	2	6
4	3	0
Total	15	370

Aggregation 'B'		
Internode generation	Rhizoid	
	Present	Absent
18		10
17		21
16		16
15		19
14		37
13		72
12		42
11		55
10		61
9		56
8	1	29
7	2	16
6	2	8
5	1	2
Total	6	444

Table 5.30 Rhizoid presence or absence by internode generation (number two and apical autozooids and 'x' and 'i' cases excluded)

Table 5.30 shows that rhizoids were concentrated vertically in the more proximal generations of the colony. (They were also concentrated horizontally, in the internodes of the long 'stem sequences', and those internodes laterally close to them).

5.3.2.3 THE DISTRIBUTION OF OVICELLS THROUGHOUT THE COLONY

In this section, ovicells were used as indicators of the distribution of autozooids producing embryos, requiring ovicells; i.e. the autozooid referred to, is that proximal to the autozooid actually producing the ovicell.

Was ovicell occurrence related to internode generation?

Internode generation	Ovicell		Percentage occurrence
	Present	Absent	
28		1	
27		14	
26		21	
25		64	
24		140	
23		202	
22		348	
21		530	
20		547	
19		710	
18	3	808	0.4
17		796	
16	13	728	1.8
15	10	559	1.8
14	18	456	3.8
13	18	485	3.6
12	21	389	5.1
11	25	337	6.9
10	21	308	6.4
9	15	301	4.7
8	11	265	4.0
7	4	163	2.4
6	2	76	2.6
5		32	
4		17	
3		8	
2		2	
1		3	
Total	161	8310	1.9

Chi-Square 221.118 P= < 0.001 (16/29)

Table 5.31 Ovicell presence or absence by internode generation (apical autozooids and 'x' and 'i' cases excluded)

Table 5.31 shows that ovicells were absent from the first five generations of internodes, occurred at a low if variable level throughout the next 13, and were completely absence from the most distal 10 generations.

[The colony was collected 15/11/97, and given that larval settlement occurs in June and July, embryo and ovicell production were probably in their early stages].

Was ovicell occurrence related to whether an internode was a 'stem' or a 'branch'?

Internode type	Ovicell		Percentage occurrence
	Present	Absent	
'stem'	87	4552	1.9
'branch'	74	3757	1.9

Chi-Square, Yates' Correction for Continuity 0.12 P= 0.851

Table 5.32 Ovicell presence or absence by 'stem' and 'branch' internodes (apical autozooids and 'x' and 'i' cases excluded)

Table 5.32 gives no indication that ovicell occurrence was related to whether an internode was a 'stem' or a 'branch'.

It is worth noting that ovicells were both rare and restricted to even-numbered autozooids. Further, no ovicells were present within the 10 long 'stem sequences'.

5.4 SUPPLEMENTARY STUDIES

5.4.1 Introduction

As discussed in Section 4.5.4 of Materials and Methods, although only the detailed studies could investigate any colony-wide aspects of a zooid's pattern of occurrence, they were not completely satisfactory. Firstly, it was desirable to look at more colonies, perhaps from more populations. Secondly, tentative conclusions had been drawn from very small samples, and it was necessary to expand the scale of these where possible. I felt, therefore, that a number of supplementary studies would be useful in an effort to address these deficiencies. These investigations did not always include all of the parameters considered in the detailed study, but were targeted at aspects requiring further investigation.

5.4.2 The distribution of unpredictably occurring polymorphs and ovicells associated with a long 'stem sequence' and the internodes which developed from it

The previous investigations showed that there was considerable variation in the level of occurrence of lateral avicularia between colonies of the Musselwick population, and between these and those of the Bay Fine population. A long 'stem sequence', together with all of the internodes which developed from it, was taken to compare, in respect of lateral avicularia, vibracula, rhizoids, and ovicells, with the colony from Musselwick, of the detailed study.

5.4.2.1 LATERAL AVICULARIA

(The distribution of lateral avicularia is shown diagrammatically in Figure 5.7).

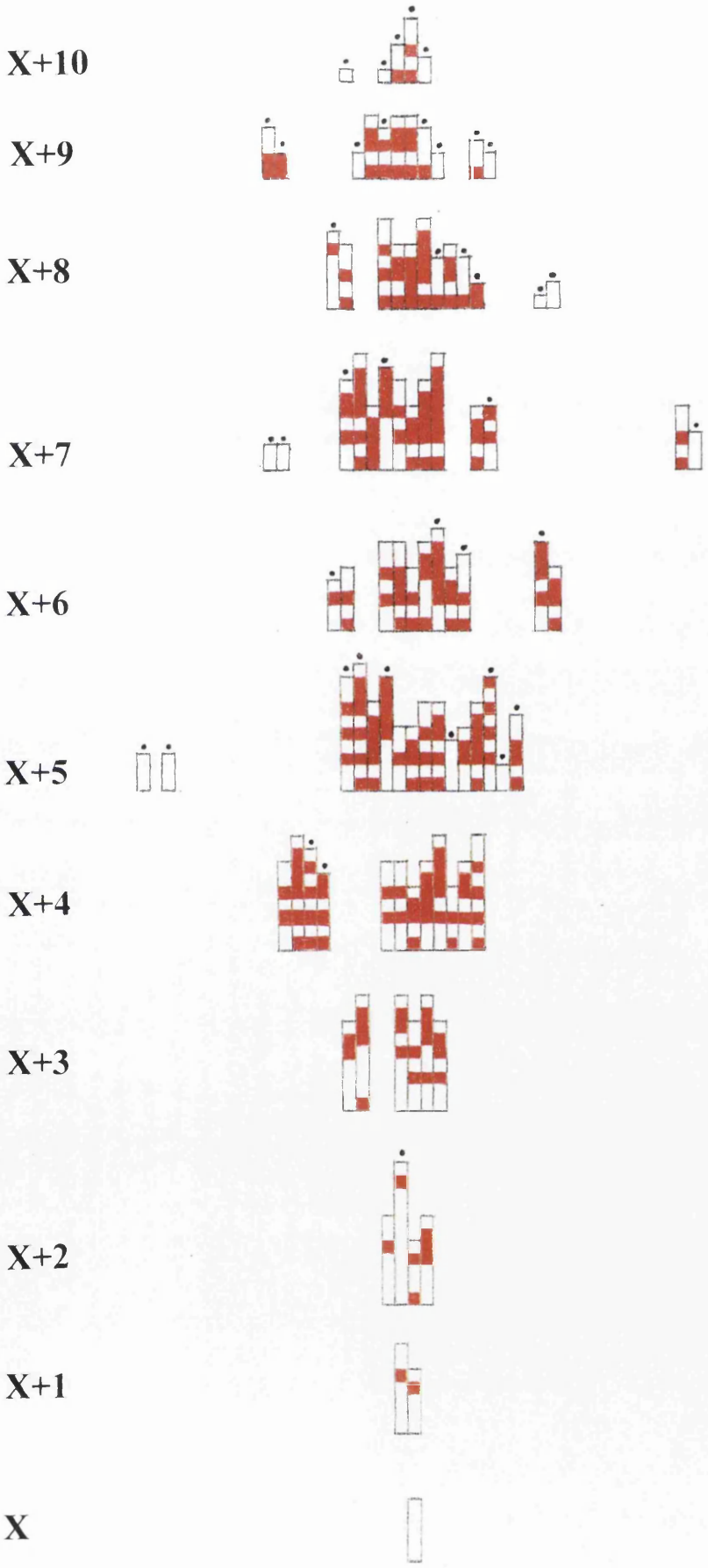


Figure 5.7 The pattern of lateral avicularia occurrence in all of the internodes which developed from a long 'stem sequence' (Swanage).

Figure 5.7 shows that in this 'aggregation' of internodes, lateral avicularia occurred on ~60% of the autozooids which could give rise to them. There was no obvious pattern of occurrence, vertically or laterally, relative to the 'aggregation' of internodes as a whole.

How did their distribution relate, compared to the colony of the detailed study, in respect of the parameters considered earlier?

Was lateral avicularia presence or absence related to internode generation?

(The material was only part of a colony and I did not know the actual generations of the internodes, hence 'X', 'X'+1...).

Internode generation	Lateral avicularia		Percentage occurrence
	Present	Absent	
X+10	3	2	60
X+9	17	6	74
X+8	26	13	67
X+7	41	19	68
X+6	31	20	61
X+5	44	31	59
X+4	41	31	57
X+3	18	18	50
X+2	7	14	33
X+1	2	7	22
X	0	3	0
Total	230	164	58

Chi-Square 21.535 P= 0.018 (5/23)

Table 5.33 Lateral avicularia presence or absence by internode generation (apical autozooids and 'x' and 'i' cases excluded)

Table 5.33 shows a steady increase in the occurrence of lateral avicularia, generation on generation.

Was lateral avicularia presence or absence related to whether an internode was a 'stem' or a 'branch'?

Internode type	Lateral avicularia		Percentage occurrence
	Present	Absent	
'stem'	126	73	63
'branch'	104	91	53
Total	230	164	58

Chi-Square, Yates' Correction for Continuity 3.639 P= 0.056

Table 5.34 Lateral avicularia presence or absence by whether an internode was a 'stem' or a 'branch' (apical autozooids and 'x' and 'i' cases excluded)

Table 5.34 shows some evidence that lateral avicularia occurrence was related to whether an internode was a 'stem' or a 'branch'.

Was lateral avicularia presence or absence related to whether an internode was complete or incomplete?

Internode type	Lateral avicularia		Percentage occurrence
	Present	Absent	
Complete	162	104	61
Incomplete	68	60	53
Total	230	164	58

Chi-Square, Yates' Correction for Continuity 1.843 P= 0.175

Table 5.35 Lateral avicularia presence or absence by whether an internode was complete or incomplete (apical autozooids and 'x' and 'i' cases excluded)

Table 5.35 shows little evidence that lateral avicularia occurrence was related to whether an internode was complete or incomplete.

Was lateral avicularia presence or absence related to autozooid position within an internode?

Autozooid position	Lateral avicularia		Percentage occurrence
	Present	Absent	
Sub-apical	86	18	82
Proximal	123	141	47
Total	209	159	57

Chi-Square, Yates' Correction for Continuity 38.169 P= < 0.001

Table 5.36 Lateral avicularia presence or absence by autozooid position within an internode (apical autozooids and 'x' and 'i' cases excluded)

Table 5.36 shows that lateral avicularia occurred very much more frequently on sub-apical than proximal autozooids.

Was lateral avicularia presence or absence related to autozooid number?

Autozooid number	Lateral avicularia		Percentage occurrence
	Present	Absent	
10	1	0	100
9	3	0	100
8	10	4	71
7	16	1	94
6	23	14	62
5	40	3	93
4	26	34	43
3	58	13	82
2	5	61	8
1	48	34	59
Total	230	164	58

Chi-Square 125.800 P= < 0.001 (4/20)

Table 5.37 Lateral avicularia presence or absence by autozooid number (apical autozooids and 'x' and 'i' cases excluded)

Table 5.37 shows that lateral avicularia occurred more frequently on the odd-numbered autozooid of each staggered pair. The extent of the difference decreased, but the rate of occurrence increased, for both odd and even numbered autozooids, as autozooid number increased. They occurred very infrequently on autozooid No. 2.

To what extent did lateral avicularia occurrence differ on odd and even-numbered autozooids?

Autozooid number	Lateral avicularia		Percentage occurrence
	Present	Absent	
Odd-numbered	165	51	76.4
Even-numbered	65	113	36.5
Total	230	164	58.4

Chi-Square, Yates' Correction for Continuity 62.216 P= < 0.001

Table 5.38 Lateral avicularia presence or absence by odd and even-numbered autozooids (apical autozooids and 'x' and 'i' cases excluded)

Table 5.38 shows that lateral avicularia occurred more than twice as frequently on odd than even-numbered autozooids.

Within the long 'stem sequence', 25 lateral avicularia were produced by 39 autozooids, a rate of occurrence of 64%, marginally higher than the level obtaining in the 'aggregation'.

5.4.2.2 VIBRACULA

(The distribution of vibracula is shown diagrammatically in Figure 5.8)

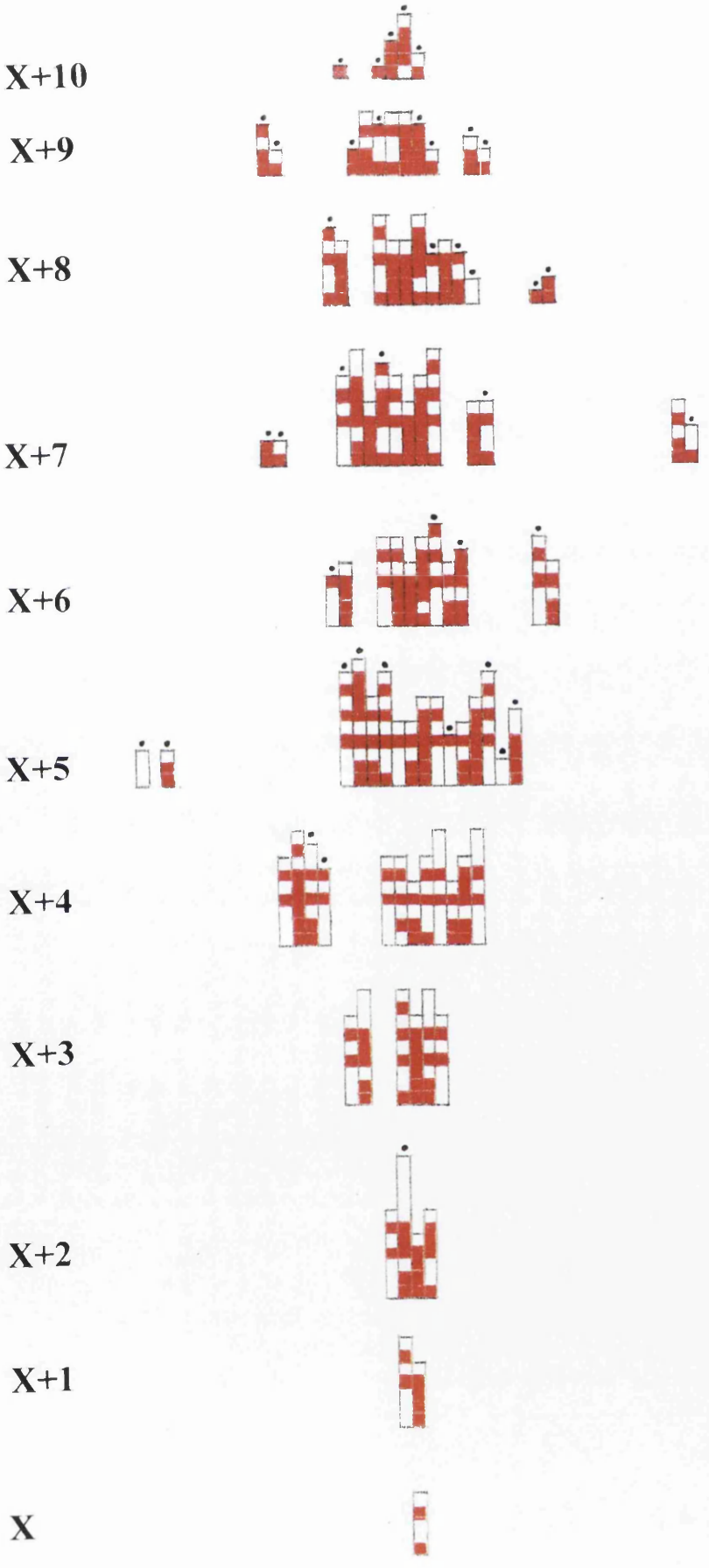


Figure 5.8 The pattern of vibracula occurrence in all of the internodes which developed from a long 'stem sequence' (Swanage).

Figure 5.8 shows that there was no obvious pattern of occurrence, vertically or horizontally, relative to the 'aggregation' as a whole.

Table 5.39 below shows that vibracula occurred on 63% of the autozooids able to produce them, a level of occurrence ~3x the level of the colony from Musselwick.

How did their pattern of occurrence relate to the parameters considered earlier?

Was vibracula presence or absence related to internode generation?

Internode generation	Vibracula		Percentage occurrence
	Present	Absent	
X+10	7	2	78
X+9	19	5	79
X+8	29	7	81
X+7	40	17	70
X+6	31	17	65
X+5	40	31	56
X+4	32	32	50
X+3	20	15	57
X+2	13	10	57
X+1	5	3	62
X	2	1	67
Total	238	140	63

Chi-Square 16.537 P= 0.085

Table 5.39 Vibracula presence or absence by internode generation (apical and number two autozooids and 'x' and 'i' cases excluded)

Table 5.39 shows that vibracula occurrence was generally constant over the generations, increasing slightly distally.

Was vibracula presence or absence related to whether an internode was a 'stem' or a 'branch'?

Internode type	Vibracula		Percentage occurrence
	Present	Absent	
'stem'	141	48	75
'branch'	97	92	51
Total	238	140	63

Chi-square, Yates' Correction for Continuity 20.976 P= < 0.001

Table 5.40 Vibracula presence or absence by whether an internode was a 'stem' or a 'branch' (apical and number two autozooids and 'x' and 'i' cases excluded)

Table 5.40 shows that vibracula occurred ~50% more frequently in 'stem' than 'branch' internodes. (But see Table 5.44).

Was vibracula presence or absence related to whether an internode was complete or incomplete?

Internode type	Vibracula		Percentage occurrence
	Present	Absent	
Complete	159	75	68
Incomplete	79	65	55
Total	238	140	63

Chi-Square, Yates' Correction for Continuity 5.998 P= 0.014

Table 5.41 Vibracula presence or absence by whether an internode was complete or incomplete (apical and number two autozooids and 'x' and 'i' cases excluded)

Table 5.41 shows that vibracula occurred somewhat more frequently in complete than incomplete internodes.

Was vibracula presence or absence related to autozooid position within an internode?

Autozooid position	Vibracula		Percentage occurrence
	Present	Absent	
Sub-apical	70	38	65
Proximal	135	75	64
Total	205	113	64

Chi-Square, Yates' Correction for Continuity .000 P= 0.926

Table 5.42 Vibracula presence or absence by autozooid position within an internode (apical and number two autozooids and 'x' and 'i' cases excluded)

Table 5.42 shows no evidence that vibracula occurrence was related to autozooid position within an internode.

Was vibracula presence or absence related to autozooid number?

Autozooid number	Vibracula		Percentage occurrence
	Present	Absent	
11	0	1	0
10	0	2	0
9	1	3	25
8	9	7	56
7	2	16	11
6	43	1	98
5	14	31	31
4	76	0	100
3	23	56	29
1	70	23	75
Total	238	140	63

Chi-Square 160.575 P= < 0.001 (6/30)

Table 5.43 Vibracula presence or absence by autozooid number (apical and number two autozooids and 'x' and 'i' cases excluded)

Table 5.43 shows that vibracula occurred considerably more frequently on even-numbered autozooids. They occurred on virtually all autozooid Nos. 4 and 6, and on >50% of No. 8; occurred on 75% of autozooid No. 1, but only one third as frequently on autozooids Nos.3 and 5, compared with their even-numbered 'partners'.

The much higher level of occurrence of vibracula on autozooid No. 1, relative to all other odd-numbered autozooids, in the preliminary study, both 'aggregations' of the detailed study, and again here, although to a lesser degree, was initially as puzzling as it was consistent. The explanation may be in the following:-

The fact that the joints between internodes cut across the proximal ends of both No. 1 autozooids in the two internodes resulting from a bifurcation creates a complication, which must be allowed for, in respect of their vibracula, in translating autozooid number into internal and external autozooid series within an internode. Following a bifurcation, the vibracula on both new No. 1 autozooids occur at the distal end of the preceding internode, one in its internal, and one in its external autozooid series. Both of these autozooids essentially occur in the external series of autozooids, in the 'new' internodes.

As a result of the consistent pattern of 'stem' and 'branch' production, the No. 1 autozooid of 'stem' internodes occurs in the internal series of the preceding internode, and the No. 1 autozooid of 'branch' internodes occurs in the external row, relative to the bifurcation (see Figure 5.3). It was, therefore a simple matter to 'reallocate' vibracula on No. 1 autozooids, to the 'actual' autozooid series in which they occurred.

The logic of the above argument also required a re-designation of the 'stem' and 'branch' location of vibracula on No. 1 autozooids. This was necessary to ascertain if 'stem' and 'branch' internodes, in themselves, were related to vibracular occurrence.

Was vibracula occurrence, in the three aggregations investigated (two in the detailed study, and one here) related to whether an internode was a 'stem' or a 'branch'?

Site	'Aggregation'	Internode type	Vibracula		Percentage occurrence
			Present	Absent	
Musselwick	'A'	'Stem'	62	139	31
Musselwick	'A'	'Branch'	46	137	25
Musselwick	'B'	'Stem'	70	188	27
Musselwick	'B'	'Branch'	47	138	25
Swanage		'Stem'	128	61	68
Swanage		'Branch'	110	79	58

Musselwick 'A', Chi-Square, Yates' Correction for Continuity 1.276 P= < 0.5
Musselwick 'B', Chi-Square, Yates' Correction for Continuity .088 P= < 0.9
Swanage, Chi-Square, Yates' Correction for Continuity 3.278 P= < 0.1

Table 5.44 Vibracula presence or absence by whether an internode was a 'stem' or a branch' (apical and number two autozooids and 'x' and 'i' cases excluded)

Table 5.44 shows that vibracula were produced more frequently in 'stem' than 'branch' internodes; by 20%, in Aggregation 'A', 8% in Aggregation B, and 17% at Swanage.

Was the occurrence of vibracula on No. 1 autozooid correlated with whether the internode was a 'stem' or a 'branch' and therefore, to whether it was as a result, produced within the internal or external series of autozooids within the preceding internode?

	'Stem', (internal)		'Branch', (external)	
	Present	%	Present	%
Musselwick (Aggregation A)	24	86	4	14
Musselwick (Aggregation B)	25	78	7	22
Swanage	47	67	23	33

Musselwick 'A', Chi-Square, Yates' Correction for Continuity 25.784 P= < 0.001
Musselwick 'B', Chi-Square, Yates' Correction for Continuity 27.564 P= < 0.001
Swanage, Chi-Square, Yates' Correction for Continuity 15.116 P= < 0.001

Table 5.45 Vibracula occurrence on number one autozooids by whether they were produced by 'stem' or 'branch' internodes, and therefore whether they occurred within the internal or external series of autozooids of the preceding internode

The results in Table 5.45 are variable, reflecting variation in level of occurrence, between the 'aggregations'. The differences would not seem ascribable to the much smaller difference in levels of occurrence in 'stem' and 'branch' internodes, shown in Table 5.44. In the two 'aggregations' of internodes from the Musselwick colony, 86% and 78% respectively of vibracula on number one autozooids were produced on 'stem' internodes, and hence within the internal series of autozooids of the preceding internode. In the material from Swanage, where there was a much higher overall level of occurrence, 67% occurred on the internal series of the preceding internode.

When all of the above were taken into account, and vibracula occurrence was related to the actual series of autozooids within an internode in which it occurred, their presence or absence was very strongly related to autozooid series within an internode. In 'detailed, Musselwick', in Aggregations 'A' and 'B' 94%, and 90%, respectively, of vibracula occurred within the internal series of autozooids within an internode, whilst in the Swanage aggregation, 74% did so.

Within the long 'stem sequence', 31 vibracula were produced by 36 autozooids, a rate of occurrence of 86%, somewhat higher than the level in the 'aggregation'.

5.4.2.3 RHIZOIDS

(The distribution of rhizoids is shown diagrammatically in Figure 5.9).

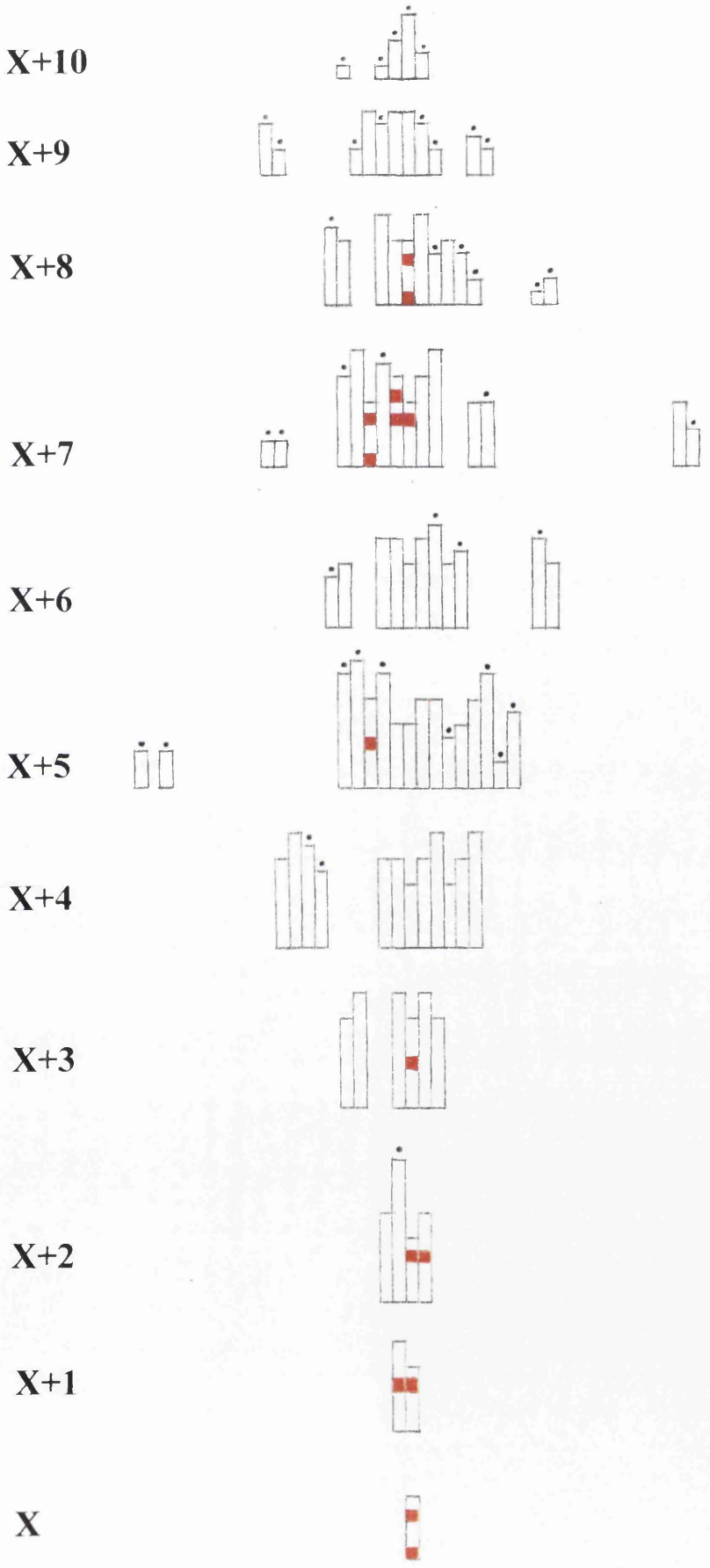


Figure 5.9 The pattern of rhizoid occurrence in all of the internodes which developed from a long 'stem sequence' (Swanage).

Figure 5.9 shows, that rhizoids were concentrated vertically within the proximal generations, and laterally, within the vicinity of the long 'stem sequence'. In this 'aggregation', they occurred firstly proximally, and then formed a second, more distal discrete cluster.

5.4.2.4 OVICELLS

(The distribution of ovicells is shown diagrammatically in Figure 5.10).

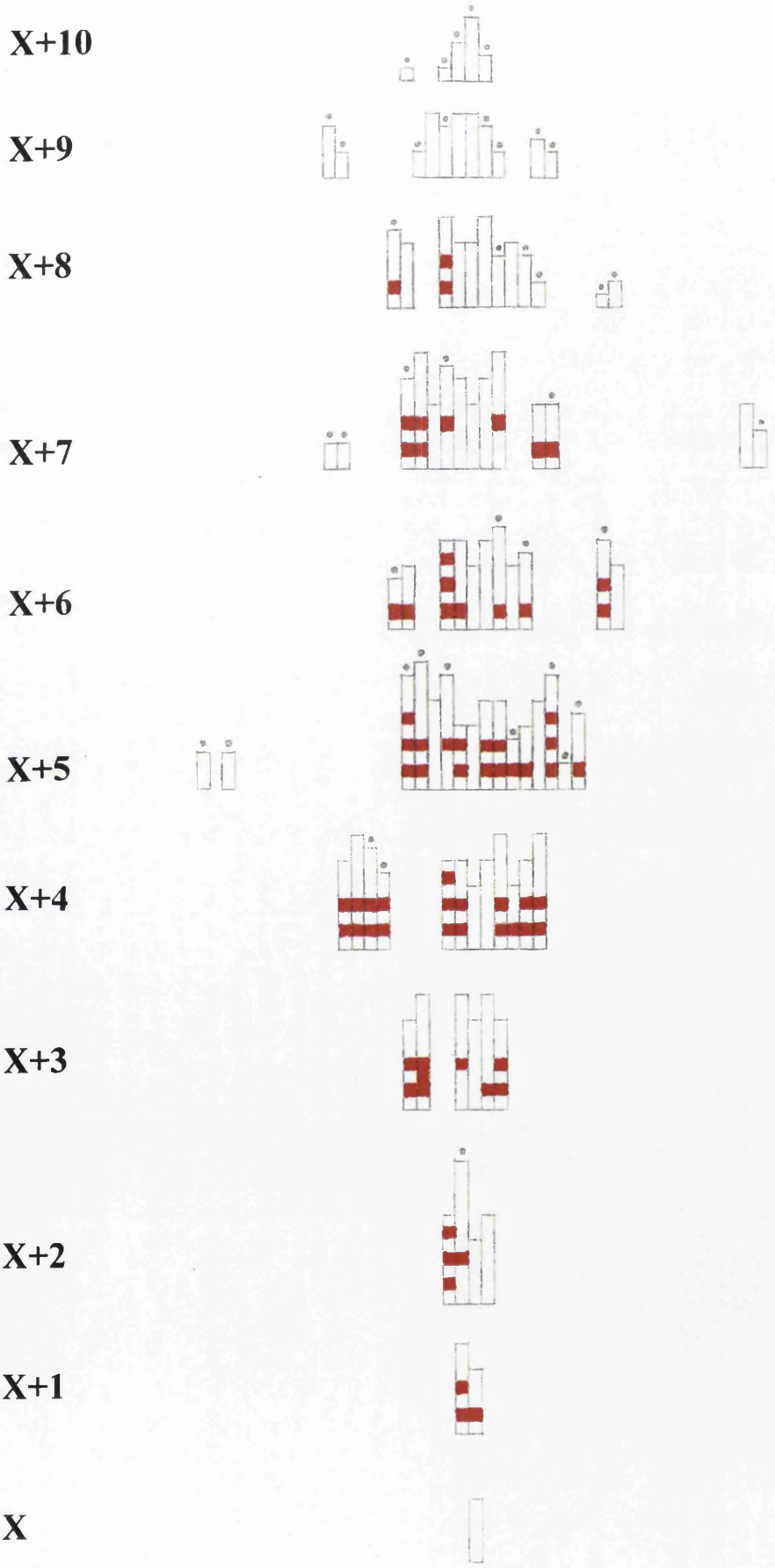


Figure 5.10 The pattern of ovicell occurrence in all of the internodes which developed from a long 'stem sequence' (Swanage).

Figure 5.10 shows no overall pattern of ovicell occurrence in relation to the 'aggregation' of internodes. They were essentially confined to even-numbered autozooids within an internode, and only one was present within the long 'stem sequence'.

Was ovicell occurrence related to internode generation?

Internode generation	Ovicell		Percentage occurrence
	Present	Absent	
X+10	0	5	0
X+9	0	23	0
X+8	3	44	6
X+7	8	63	11
X+6	10	49	17
X+5	18	71	20
X+4	20	54	27
X+3	9	33	21
X+2	4	23	15
X+1	3	7	30
X	0	4	0
Total	75	376	17

Chi-Square 20.070 P= 0.029 (7/32)

Table 5.46 Ovicell presence or absence by internode generation (apical autozooids and 'x' and 'i' cases excluded)

Table 5.46 shows, beyond the fact that this 'stem sequence' did not originate in one of the earliest generations, that ovicell production was not noticeably related to internode generation, and when it did occur, that it was at a generally low level.

Was ovicell occurrence related to whether an internode was complete or incomplete?

Internode type	Ovicell		Percentage occurrence
	Present	Absent	
Complete	49	239	17.0
Incomplete	26	137	16.0
Total	75	376	16.6

Chi-Square, Yates' Correction for Continuity .025 P= 0.873

Table 5.47 Ovicell occurrence by whether an internode was complete or incomplete (apical autozooids and 'x' and 'i' cases excluded)

Table 5.47 gives no indication that ovicell occurrence was related to whether an internode was complete or incomplete.

Was ovicell occurrence related to whether an internode was a 'stem or a 'branch'?

Internode type	Ovicell		Percentage occurrence
	Present	Absent	
'Stem'	27	199	11.9
'Branch'	48	177	21.3
Total	75	376	16.6

Chi-Square, Yates' Correction for Continuity 6.504 P= 0.011

Table 5.48 Ovicell presence or absence by 'stem' and 'branch' internodes (apical autozooids and 'x' and 'i' cases excluded)

Table 5.48 shows that ovicells occurred almost twice as frequently on 'branch' than 'stem' internodes.

Was ovicell occurrence related to autozooid position within an internode?

Autozooid position	Ovicell		Percentage occurrence
	Present	Absent	
Sub-apical	4	104	3.7
Proximal	71	217	24.7
Total	75	321	18.9

Chi-Square, Yates' Correction for Continuity 21.109 P= < 0.001

Table 5.49 Ovicell presence or absence by autozooid position within an internode (apical autozooids and 'x' and 'i' cases excluded)

Table 5.49 shows that ovicells were rarely produced in association with sub-apical autozooids.

Was ovicell occurrence related to odd or even–numbered autozooids?

Autozooid number	Ovicell		Percentage occurrence
	Present	Absent	
Odd-numbered	1	230	0.4
Even-numbered	74	145	33.8
Total	75	375	16.7

Chi-square, Yates' Correction for Continuity 87.678 P= < 0.001

Table 5.50 Ovicell presence or absence by odd and even-numbered autozooids (apical autozooids and 'x' and 'i' cases excluded)

Table 5.50 shows that ovicells occurred virtually only on even-numbered autozooids.

All 75 ovicells, including the one on an odd-numbered autozooid, had a vibraculum adjacent to them.

Within the long 'stem sequence' one ovicell was produced by 45 autozooids, a rate of occurrence of 2.2%, less than $1/6^{th}$ the level obtaining in the 'aggregation'.

5.4.3 Possible delayed development of polymorphic zooids

Because the coded recording scheme did not facilitate the investigation of the distribution of polymorphic zooids in relation to branch tips, this was carried out in a separate study. A number of branch tips were examined, and it was assumed that a series of different scuta morphologies present, working back from the branch tip (related to ontogenetic stages of growth) indicated that growth was occurring, or had been stopped when the colony was collected. It certainly enabled damaged branches to be distinguished.

For frontal and lateral avicularia, and vibracula, there was evidence of their incipient formation up to the distalmost autozooid. Rhizoids were not found within several internodes of growing tips, except in very small colonies.

5.4.4 Production of ovicells in relation to autozooid number, frontal avicularia occurrence and autozooid position within an internode; and whether an internode was complete or incomplete

The main 'stem sequences' investigated in the preliminary study were completely without ovicells. The large colony chosen for the detailed investigation had very few, and all of these were on even-numbered autozooids; and frontal avicularia and ovicells were mutually exclusive. It was therefore, desirable to investigate a more fertile colony, and 300 ovicellate internodes from this were investigated (see Section 4.5.5.1.3). (Raw data are in Appendix 'I').

Was ovicell occurrence related to autozooid number?

Autozooid number	Ovicell		Percentage occurrence
	Present	Absent	
14	0	4	0
13	0	4	0
12	1	6	14
11	1	6	14
10	7	7	50
9	1	14	7
8	15	30	33
7	1	51	2
6	75	114	40
5	3	200	2
4	208	74	74
3	11	282	4
2	254	46	85
1	0	299	0
Total	577	1137	34

Table 5.51 Ovicell presence or absence by autozooid number in 300 ovicellate internodes of one colony (apical autozooids and 'x' and 'i' cases excluded)

Table 5.51 shows, that only 17 of the 577 ovicells, 3%, were produced by odd-numbered autozooids. Those odd-numbered autozooids which did produce ovicells, also produced frontal avicularia. This was achieved by a slight reduction in the size of the ovicell, a greater reduction in the size of the frontal avicularium, and the fact that the latter appeared to be positioned slightly more centrally within the internode.

Was ovicell presence or absence related to autozooid position within an internode?

Autozooid position	Ovicell		Percentage occurrence
	Present	Absent	
Sub-apical	59	347	15
Proximal	512	771	40
Total	571	1118	34

Chi-Square, Yates' Correction for Continuity 87.604 P= < 0.001

Table 5.52 Ovicell presence or absence by autozooid position within an internode (apical autozooids and 'x' and 'i' cases excluded)

Table 5.52 shows that ovicells occurred almost three times more frequently on proximal than sub-apical autozooids.

Was ovicell occurrence related to whether an internode was complete or incomplete?

Internode type	Ovicell		Percentage occurrence
	Present	Absent	
Complete	411	827	33
Incomplete	165	293	36
Total	576	1120	34

Chi-Square, Yates Correction for Continuity 1.069 P= < 0.9

Table 5.53 Ovicell presence or absence by whether an internode was complete or incomplete (apical autozooids and 'x' and 'i' cases excluded)

Table 5.53 shows no evidence that ovicell occurrence was related to whether an internode was complete or incomplete.

5.4.5 Unquantified investigations

An inevitable consequence of the detailed nature of the two detailed studies was that only a limited range of material was investigated. There was concern that variation in level or pattern of occurrence could well occur between populations, or between colonies, and go unrecorded. The supplementary studies were carried out to address this, but ideally a still greater range of material would have been investigated.

A number of unrecorded investigations were therefore made, in respect of two aspects.

- Firstly, to confirm, or refute, the general occurrence of a characteristic, present in all of the material investigated.
- Secondly, where there was evidence of variation, to try to establish whether this occurred at the level of the population, or that of the colony.

Four separate areas were investigated:-

- Firstly, there was the whole question of the existence of a limited number of long 'stem sequences', with lateral growth centred on them, within a colony. Main 'stem sequences' were apparent in all 30 colonies of the preliminary study. A main, and a small additional number of long 'stem sequences', were present in 'detailed Musselwick', and 'supplementary Swanage', each of which were single colonies. I looked at a number of well-developed colonies from all three populations, and in all, the existence of a limited number of long 'stem sequences', and distally, largely discrete aggregations of internodes, within short 'stem sequences', associated with them, were characteristic.
- Secondly, the colonies of the five main 'stem sequences' of colonies from Musselwick, in the preliminary investigation, were examined to determine whether or not the colonies differed, in respect of level of lateral avicularia occurrence, to the same extent as their main 'stem sequences'. The level of lateral avicularia occurrence was low in all five colonies, and although less variable than in the main 'stem sequences', there was evidence of inter-colony variation.
- Thirdly, the single 'aggregation' of internodes investigated from the Swanage population, exhibited a level of lateral avicularia occurrence, nearly four times higher than that of the Musselwick material. An investigation of 'aggregations' from six further colonies from this population, revealed a similar high level of lateral avicularia occurrence in all six.
- Finally, the single aggregation of internodes investigated from the Swanage population, exhibited a level of vibracula occurrence twice the level of that of the Musselwick material. An investigation of aggregations from six further

colonies from this population, revealed a similar high level of vibracula occurrence in all six.

The above, whilst not conclusive, strongly suggest, that a limited number of long 'stem sequences' with, distally, areas of growth occurring only in relation to them, were characteristic of all three of the populations investigated. Further, that in respect of the variation in the aspects described above, the material investigated was generally typical of its population. Only at Musselwick, in respect of the level of lateral avicularia occurrence, was there evidence of inter-colony variation. This casts considerable doubt on the validity, for lateral avicularia, of speaking of inter-population variation. The level of vibracula occurrence at Swanage, in the material investigated, was twice that obtaining at Bay Fine and Musselwick, strongly suggesting inter-population variation. For unpredictably occurring polymorphs, variation in level of occurrence is not unexpected. It is also true, that while spatial patterns of occurrence, were less pronounced at high levels of occurrence, they did not change in character, nor disappear.

5.5 COLLATION AND SUMMARY OF RESULTS

5.5.1 Introduction

To avoid unnecessary repetition, the full discussion of the results of this Chapter and of Chapter 6 will be contained in Chapter 7. In this collation and summary I shall bring together the results from the preliminary, detailed and supplementary studies, make clear the extent of any variation between them, and summarise the results characteristic by characteristic.

A major concern at the outset was that inter-colony, or inter-population, variation could militate against the making of statements applicable to the species. Bringing together the results from the various studies gives some idea of which characteristics occurred consistently, and which did not. There was, however, little initial evidence in the preliminary study of such variation, in respect of autozooids, internodes, 'stem sequences', or the majority of the polymorphs. The one exception was the level of lateral avicularia occurrence, which differed, for the main 'stem sequences', both between the colonies at Musselwick, and also between the Musselwick, and Bay

Fine, populations. A subsequent supplementary study of a colony from Swanage, revealed a much higher level of lateral avicularia and vibracula occurrence, than occurred at Musselwick.

This collation and summary of results can conveniently be divided into two sections. The first, initially concerned with the arrangement of autozooids within internodes, bifurcations, complete and incomplete internodes, and 'stems' and 'branches'. This leads to internodes within 'stem sequences', 'stem sequences' within the colony, and colony structure and form. The second, relating to the numbers and spatial arrangement of polymorphic heterozooids, and the pattern of reproductive zooids, as evidenced by the presence or absence of ovicells, within the colony.

The preliminary study was concerned solely with the main 'stem sequences' of a number of colonies from populations at Musselwick, Pembrokeshire, and Bay Fine, I.O.M. The detailed study was carried out on a single colony from Musselwick. Supplementary studies, to augment the above, were carried out on material from Swanage, Dorset. In order to minimise the wordiness of the references to these in what follows, I shall refer to them as 'preliminary, Musselwick'; 'preliminary, Bay Fine'; 'detailed, Musselwick'; and 'supplementary, Swanage'.

5.5.2 Autozooids in internodes, bifurcations, internodes within 'stem sequences', their arrangement within the colony, and its structure and form

5.5.2.1 AUTOZOOIDS WITHIN INTERNODES, AND BIFURCATIONS

Internodes were distinguished in three respects, the number of their constituent autozooids, whether they were complete or incomplete, and whether they were a 'stem' or a 'branch'.

The arrangement of autozooids within the first internode of a colony is different from that in all other internodes. The ancestrula is rarely present, except in very young colonies, and the number of autozooids present, excluding it, may be an odd or even number (generally three or four). The distalmost three autozooids are always

arranged as in all other internodes (which are always of an odd-number of autozooids) (Lutaud, 1953).

Within the main 'stem sequences' of the preliminary study, the overwhelming majority of internodes were of five autozooids, with only the first internode of each being generally of three or four, and the second, of three autozooids (see Table 5.1). Although not quantified, it was clear that outside these main 'stem sequences', where only four internodes (1.5%) were of seven autozooids, such internodes were probably the most numerous length. Within the colony of 'detailed Musselwick', the very first internode of the colony was of four, the next two were of three, and all other internodes were of an odd-number of autozooids (Table 5.7). Of these, the majority, >90%, were of five or seven; internodes of seven outnumbering those of five by three to two; and there were a minority of nine, and a small number of 11 and 13 (Table 5.8).

Internodes, on completing their growth, bifurcate and initiate two more. Each bifurcation is asymmetrical; the parent internode is essentially continued by the production of a primary ramus, or 'stem', the direction of growth of which deviates little from that of its precursor. A secondary ramus, or 'branch', is produced slightly more proximally, and deviates from the original direction of growth at a greater angle. Because all internodes consist of an odd-number of autozooids and each bifurcation is asymmetrical, each bifurcation, if one progresses from 'stem' to 'stem', is a mirror image of its predecessor. The consistent pattern of branching which obtains throughout a colony results from this (Lutaud, 1953).

The finite space available for colony growth means that not all internodes reach maturity and bifurcate. Such 'incomplete' internodes constituted some 40% of the internodes of the colony of 'detailed Musselwick'.

Tables 5.9 and 5.10 showed that, in 'detailed, Musselwick', there were more 'stems' than 'branches', 17.5% and 21%, more for complete and incomplete internodes, respectively. Also the longer a complete internode, the more likely it was to be a 'stem'. In terms of occurrence and length, 'stems' and 'branches' did differ.

Distinguishing between 'stem' and 'branch' internodes led to the concept of 'stem sequences', which was central to describing the arrangement of internodes within a colony.

5.5.2.2 INTERNODES WITHIN 'STEM SEQUENCES'

'Stem sequences' are sequences of internodes, which deviate from their predecessor and successor by the smaller of the two angles possible.

The preliminary investigation revealed, although not quantitatively, that the internode composition of these main 'stem sequences', was very different from that obtaining in the colonies. In the former the vast majority of the internodes were of five autozooids (Table 5.1), whilst in the colonies, internodes of seven autozooids were very common, probably the most numerous length.

In 'detailed Musselwick', there were a very small number of long, and a large number of very short, or short, 'stem sequences', (Table 5.12). 90% of 'stem sequences' were of five or fewer internodes, and only 3% were of 16 or more.

The 10 longest 'stem sequences' of 'detailed Musselwick' (and the main 'stem sequences' of the preliminary study) were constituted almost completely of internodes of five autozooids. A very small number of internodes of seven autozooids were present, but no longer internodes. There was no obvious sequence to their occurrence.

There was a possibility that the particular internode composition of the main, and long 'stem sequences', was a secondary feature, resulting from two known differences between them and the colony. Firstly the proportions of the different internode lengths, in the various generations of internodes, were very different in the two. Secondly, the main and long 'stem sequences', were composed almost entirely of 'stem' internodes, whilst in the colony, there were only slightly more 'stems' than 'branches'. In Section 5.3.2.1.4.3, it was demonstrated that neither of these characteristics could be invoked to explain the different internode composition of the main and long 'stem sequences'.

The internode composition of the long 'stem sequences' does not result from other characteristics of the spatial arrangement of internodes, it is a primary feature.

In 'detailed Musselwick' (Table 5.13) internodes of more than five autozooids were largely confined to the proximal positions within a 'stem sequence'. Beyond the third internode within a 'stem sequence' only 10% were of more than five autozooids, although such internodes constituted 54% of the total.

5.5.2.3 THE ARRANGEMENT OF INTERNODES AND 'STEM SEQUENCES' WITHIN A COLONY, AND THE STUCTURE AND FORM WHICH RESULTS

Within 'detailed Musselwick', all of the long 'stem sequences' originated in the first six generations of internodes, although short 'stem sequences' also originated in generations four, five, and six. From generation seven on, the 'stem sequences' were overwhelmingly (94%) of four or less internodes.

It was established in Section 5.3.2.1.4.4, that the 10 longest 'stem sequences' were well, if not evenly spaced within the colony.

The detailed study showed (Table 5.6) that although the potential ability of the colony to double the number of internodes, and therefore its number of growing points with each successive generation of internodes, was initially exploited, it ceased to be so in the sixth generation. For the next 10 generations, the number of internodes increased modestly, at a steadily decreasing rate, and beyond that their numbers fell.

Complete internodes showed a pattern of increase and decrease in number over the generations. Incomplete internodes were completely absent from the first six generations, and their number, as a proportion of the total number within a generation, essentially increased generation on generation.

The actual spatial distribution of all of the internodes in the half of the colony of the detailed study was represented diagrammatically (see Figure 5.4). Beyond the proximal region of the colony, where all possible internodes did develop, as growth

proceeded, there was a large scale 'thinning out', which increased in magnitude in the more distal regions. In the central and distal regions of the colony, this resulted in discrete zones of extensive vertical growth, and at the centre of each, a long 'stem sequence'.

The vast majority of the internodes of such an 'aggregation', diagrammatically represented in two dimensions, occurred in a vertical lanceolate band. The number of internodes increased, and then decreased, from one generation to the next. A very small minority of internodes formed discrete thin 'arms', which diverged widely from this central core.

The different length internodes exhibited a complex if ill-defined pattern of occurrence vertically within the colony (Table 5.7) in that the longer the length of internode the more distal, in terms of internode generation, was its first appearance, and the more proximal its disappearance. This pattern probably largely results from the arrangement of different internode lengths within 'stem sequences' and their changing numbers in relation to internode generation. Internodes longer than five autozooids were essentially restricted to the most proximal positions within their 'stem sequence'. Laterally, internodes of five autozooids were concentrated within the long 'stem sequences'.

Incomplete internodes vertically increased as a proportion of the total number of internodes, generation on generation. Laterally, they exhibited no discernible pattern, beyond their more limited occurrence, close to long 'stem sequences'.

The limited number of very long 'stem sequences' and, beyond the more proximal generations of internodes, the very strongly clumped distribution of internodes in association with them, is shown in Figures 5.4 and 5.5. It resulted in a limited number of vertically extensive, laterally limited, 'aggregations' of internodes, which distally were laterally discrete. Each 'aggregation', centred on a long 'stem sequence', was, in two dimensions lanceolate shaped.

In actuality an 'aggregation' of internodes developing from a long 'stem sequence', is not two-dimensional. The angulation of internodes in relation to one another at bifurcations, and to a lesser extent, the concave nature, lengthwise, of the longer

internodes, results in incurving, both distally and laterally of the 'aggregation'. The three-dimensional overall structure of the colony which results, is essentially an incomplete circle of discrete, slender, incomplete 'flasks', which develop from a vertically limited, laterally continuous, proximal region.

5.5.3 The spatial arrangement of polymorphs and ovicells

5.5.3.1 INTRODUCTION

The results of the investigation into the spatial arrangement of polymorphic heterozooids and ovicells were more complicated than those of autozooids and internodes. Firstly, this was because there was evidence, for two of the polymorphs, of inter-population, and perhaps, inter-colony, variation. Secondly, because there were a greater number of parameters to consider, the influences of which, were not necessarily, independent of one another. Polymorph and ovicell pattern of occurrence can, indeed initially has to, be described by reference to their observed spatial distribution. A polymorph's occurrence may be described relative to certain parameters of characteristic elements of the colony, or their spatial disposition, on various scales, within the colony. There may be difficulties in establishing the actual scale at which some of these distributions occur.

As will be discussed in Chapter 7, the observed spatial arrangement of polymorphs and ovicells, whilst it may include intrinsically spatially determined elements, is likely to result from the combined affects of several factors. Correlations, positive and negative between polymorphs, or between a polymorph and an ovicell, may well exert an influence, as may the overall level of occurrence. I shall consider each of these in turn but it is important that the various aspects of the pattern of occurrence actually observed are not viewed as inevitably occurring independently of one another. For convenience they will be considered in the following order:-

- The observed spatial pattern of polymorphs and ovicells, in relation to the colony or smaller constituent units within it.
- The various apparent correlations, positive or negative, between polymorphs, or between polymorphs and ovicells.
- The level of polymorph occurrence.

5.5.3.2 THE SPATIAL ARRANGEMENT OF POLYMORPHS AND OVICELLS WITHIN A COLONY

Patterns of spatial occurrence of polymorphs within a colony, on whatever scale, can be ascribed to one of three categories, in relation to whether they occurred constantly, predictably or unpredictably.

Mural spines and scuta occurred absolutely constantly on all autozooids, except the ancestrula, in a constant fashion, as has long been known. Likewise a single vibraculum occurred in each branch axil.

5.5.3.2.1 Frontal avicularia

Large frontal avicularia occurred in an absolutely predictable fashion, except on the first internode of a colony, on all odd-numbered autozooids, including the apical autozooid, but excluding number one; and on no even-numbered autozooids.

The remaining polymorphs, lateral avicularia, vibracula and rhizoids, and the ovicells, all occurred unpredictably, although not equally so! Although I had anticipated that their occurrence could relate to the 'aggregations' of internodes associated with long 'stem sequences' none exhibited any such pattern in relation to them.

5.5.3.2.2 Lateral avicularia

For lateral avicularia, in 'preliminary Musselwick' there was considerable variation in the level of occurrence, between the main 'stem sequences', of the five colonies. The overall level of lateral avicularia occurrence, within the main 'stem sequences', was five times higher at Bay Fine than at Musselwick. The level of occurrence in the two 'aggregations' of 'detailed Musselwick' were slightly different, but that in 'supplementary Swanage' was on average ~3 times greater, and all asymmetries of occurrence were much reduced.

- Lateral avicularia exhibited a decidedly clumped distribution.
- Lateral avicularia occurred approximately twice as frequently in long 'stem sequences' than outside them in 'detailed Musselwick, but only marginally so in 'supplementary Swanage'.
- Lateral avicularia occurrence exhibited no relation to internode generation in 'detailed Musselwick'; but the level of occurrence increased, generation on generation, in 'supplementary Swanage'.
- Lateral avicularia occurred somewhat more frequently in 'stem' than 'branch' internodes, in 'detailed Musselwick, and in 'supplementary Swanage'.
- Lateral avicularia occurred three to four times more frequently in complete than incomplete internodes, in 'detailed Musselwick'; but only marginally more frequently in 'supplementary, Swanage',
- Lateral avicularia occurred 2–3.5x more frequently on sub-apical, than proximal autozooids, in 'detailed Musselwick', but only twice as frequently in 'supplementary Swanage'.
- Lateral avicularia occurrence, in relation to autozooid number, showed a similar pattern of occurrence in 'detailed, Musselwick', and 'supplementary Swanage'. They occurred more frequently, ~2.5x, on the odd-numbered autozooid of each staggered pair, and, in 'supplementary Swanage' the frequency of occurrence generally increased as autozooid number increased. They occurred most frequently on autozooids three and five and rarely on autozooid number two. The three times higher level of occurrence at Swanage blurred the pattern of occurrence.
- The large forms were invariably only found on the external autozooid of the sub-apical pair, in all of the material studied.

5.5.3.2.3 Vibracula

The level of vibracula occurrence in the preliminary investigation was very similar in the main 'stem sequences' of colonies from Musselwick and Bay Fine, at ~66%. The level in the colony, of 'detailed Musselwick', was much lower at ~27%, although the level of occurrence within the long 'stem sequences' was ~70%, very similar to the level obtaining in the main 'stem sequences', of the preliminary study. The level of occurrence in 'supplementary Swanage', of 63%, was more than twice that at

Musselwick. The level in the long 'stem sequence' here, was also higher, at 91%. The >2 times higher level of occurrence in 'supplementary Swanage' resulted in all asymmetries being much reduced.

- Vibracula occurred more frequently in long 'stem sequences' than outside them, 2.5 and 3 x in the two 'aggregations'.
- Vibracula occurred at a high level in the early generations, but then fell to a much lower level, in 'detailed Musselwick'. In 'supplementary Swanage' the level of occurrence was consistently high.
- Vibracula occurred ~3, and ~7 x, more frequently in complete internodes, in the first and second 'aggregations' respectively in 'detailed Musselwick'; but only 25% more frequently in 'supplementary Swanage'.
- Vibracula occurred 50% more frequently in 'stem' than 'branch' internodes in Aggregation 'A' from Musselwick, and in 'supplementary Swanage'. They occurred only 25% more frequently in Aggregation 'B'.
- Vibracula occurrence was not related to autozooid position within an internode in 'detailed Musselwick', nor in 'supplementary Swanage'.
- Vibracula were rare on odd-numbered autozooids, except autozooid number one, in both 'aggregations' in 'detailed Musselwick'. The situation was very similar in 'supplementary Swanage', but less clear-cut.

Their much higher level of occurrence on autozooid number one, relative to all other odd-numbered autozooids, in all of the material in all of the studies, was investigated in Section 5.4.2.2. Essentially this involved reallocating the vibracula on number one autozooids, which actually occur within the preceding internodes; to the actual autozooid series, internal or external, within the internode in which they occurred, relative to the preceding bifurcation. This adjustment revealed that in all of the material:-

- Vibracula were concentrated in the internal series of autozooids. In 'detailed Musselwick', in Aggregations 'A' and 'B', 94% and 90%, respectively, of vibracula occurred within the internal series of autozooids within this, whilst in the 'aggregation' of 'supplementary Swanage', 74% did so.

It is worth noting that axial vibracula, excluded from the general analysis, occurred on all No. 2 autozooids in stem internodes and were absent from all such internodes in branches. They thus occurred on 100% of stems, were absent from all branches, and occurred on 50% of No. 2 autozooids.

5.5.3.2.4 Rhizoids

Rhizoids were concentrated vertically, as has long been known, in the more proximal generations of internodes. They also exhibited a definite lateral spatial arrangement, being concentrated within, or close to, long 'stem sequences'.

5.5.3.2.5 Ovicells

- In 'detailed Musselwick' no ovicells were produced in the first five generations of internodes, they were produced at a very low, but variable level, over the next 10 generations, and none were produced in the distalmost generations. The situation was very similar in 'supplementary Swanage'.
- In 'detailed Musselwick', all of the few ovicells produced were on even-numbered autozooids. In more fertile material in 'supplementary Swanage', 97% of ovicells were produced by even-numbered autozooids.
- Ovicells were absent from the main 'stem sequences' of 'preliminary Musselwick' and the long 'stem sequences' of 'detailed Musselwick'. In more fertile material in 'supplementary Swanage', a small number of ovicells were produced within long 'stem sequences'.
- Ovicells occurred almost three times more frequently on proximal than sub-apical autozooids, in 'supplementary Swanage'.
- Ovicell occurrence was not related to whether an internode was complete or incomplete in 'supplementary Swanage'.
- Ovicells occurred twice as frequently on branches than stems in 'supplementary Swanage'.

5.5.3.3 POSITIVE AND NEGATIVE CORRELATIONS BETWEEN VARIOUS POLYMORPHS, AND BETWEEN POLYMORPHS AND OVICELLS

These were observed correlations, which may not, of course, be due solely to positive or negative associations between polymorphs, or between polymorphs and ovicells.

5.5.3.3.1 Frontal avicularia and vibracula.

In 'detailed Musselwick', frontal avicularia and vibracula were virtually mutually exclusive, with the former occurring on all odd-numbered autozooids, except number one, and the latter being restricted, almost entirely, to even-numbered autozooids, plus number one. (However, see remarks regarding vibracula on number one autozooids in Section 5.5.3.2.3). In 'supplementary Swanage', the overall level of vibracula occurrence was more than twice the level obtaining at Musselwick, and the two polymorphs were, inevitably, less mutually exclusive.

5.5.3.3.2 Frontal avicularia and ovicells

Frontal avicularia and ovicells occupy a very similar position on the gymnocyst proximal to the frontal membrane. The main 'stem sequences' of all of the colonies of the preliminary study were without ovicells, and the colony of the detailed study had very few, all of which occurred on even-numbered autozooids, which were always without frontal avicularia. A supplementary study of a number of internodes, containing ovicellate autozooids, from a very fertile colony at Swanage revealed that only 17 of 577 ovicells, 3%, were produced by odd-numbered autozooids, and that these autozooids also produced frontal avicularia. This was achieved by a reduction in the size of both avicularium and ovicell, and, an apparent slight shift in the location of the former. Frontal avicularia and ovicells, were therefore, not absolutely mutually exclusive, but very largely so.

5.5.3.3.3 Vibracula and ovicells

Vibracula occurred far more frequently on the autozooids within the internal series of autozooids. This was more apparent in 'detailed Musselwick' than 'supplementary

Swanage' and here ovicells occurred almost exclusively on autozooids, in the internal series of autozooids. Vibracula were more numerous than ovicells, and, for all of the material investigated in this respect, each ovicell had a vibracula sited adjacent to it.

5.5.3.3.4 Vibracula and rhizoids

Rhizoids, where they do occur, develop from vibracula, and cannot develop in their absence. There was evidence that vibracula occurred more frequently within long 'stem sequences', than outside them, and conceivable that this was related to a requirement for rhizoids, which were concentrated laterally within them.

5.5.3.4 THE LEVEL OF POLYMORPH OR OVICELL OCCURRENCE

There was consistent evidence, throughout Sections 5.5.3.2 and 5.5.3.3, that pronounced patterns of polymorph presence or absence, very apparent at low levels of occurrence, persisted, but much less well defined, at higher levels. This is, depending on the level of occurrence, to some degree mathematically inevitable, and does nothing to suggest that the pattern observed is without significance.

5.5.4 Summary

There was no indication of any variation between colonies or populations, in respect of the arrangement of autozooids within internodes, internodes within 'stem sequences', or 'stem sequences' within a colony.

Internodes, except perhaps the first of a colony, consisted of an odd-number of autozooids, resulting in asymmetrical bifurcations, and a consistent branching pattern (Lutaud, 1953). Spatial constraints meant that not all internodes bifurcated, 40% of internodes were incomplete. Differentiating between 'stems' and 'branches' at bifurcations, led to the concept of 'stem sequences', sequences of 'stems'. A colony consisted of a small number of very long, and a large number of much shorter, 'stem sequences'. All 'stem sequences' develop in a shallowly sinusoidal manner, with an essentially constant direction of growth, and with 'branches' developing alternately

to left and right. The considerable lateral variation in the extent of vertical growth, even in the absence of external constraints, was clearly related to the existence of a limited number of long 'stem sequences', with growth, beyond the more proximal region, only developing, directly or indirectly, from them. Long 'stem sequences' differed from the shorter lengths comprising the mass of the colony, in that their constituent internodes were generally shorter, with the longest lengths being completely absent. The spatial arrangement of the various length internodes, within a colony, exhibited an indistinct pattern vertically. In general, the longer the length of internode the more distal, in terms of internode generation, its first appearance, and the more proximal its disappearance. This pattern largely results from the concentration of internodes longer than seven autozooids in the proximal positions within 'stem sequences', and their changing numbers in relation to internode generation. Laterally, internodes of five autozooids were concentrated within long 'stem sequences'.

Long 'stem sequences', laterally well-spaced, formed central ribs of lanceolate 'aggregations' of internodes (within short 'stem sequences'), which, in the central and distal regions of the colony, were quite discrete. Each 'aggregation' was incurved, distally and laterally, and the resultant colony form, was an incomplete circle of incomplete narrow flasks, developing from a vertically limited, laterally continuous, proximal region.

Autozooids are arranged, within internodes, within 'stem sequences', within a colony, in a characteristic, if not mathematically precise fashion.

There was, in respect of polymorph occurrence, evidence of both inter-colony, and inter-population variation. The level of lateral avicularia occurrence probably varied between colonies of the Musselwick population, and certainly varied between the Bay Fine and Swanage populations; and that at Musselwick. The level of vibracula occurrence was very similar in the Bay Fine, and Musselwick, populations, but was much higher in the material from Swanage.

Polymorphs occur absolutely constantly, absolutely predictably, or unpredictably. As was well-known, mural spines, scuta and axial vibracula, occurred absolutely

constantly; the first two, on all autozooids except the ancestrula, and the third in each branch axil. Frontal avicularia occurred absolutely predictably, only on odd-numbered autozooids, but not on autozooid No. 1. Lateral avicularia, vibracula, and rhizoids, occurred unpredictably. The spatial disposition of these can be described in respect of various parameters within a colony. Lateral avicularia tended to occur on odd-numbered, and sub-apical autozooids, within an internode. They occurred somewhat more frequently in 'stems' than 'branches', and much more frequently in complete than incomplete internodes. Vibracula occurred much more frequently on autozooids within the internal series of autozooids within an internode; and also much more frequently in complete internodes. Rhizoids were concentrated vertically in proximal generations, and laterally in, or close to, long 'stem sequences'. Ovicells occurred almost exclusively on even-numbered autozooids, rarely on sub-apical autozooids, and infrequently within long 'stem sequences'. Various positive and negative associations were observed between polymorphs, and between polymorphs and ovicells. Frontal avicularia were negatively correlated with both vibracula and ovicells, and these two were positively correlated with each other, as were vibracula and rhizoids; the latter developing from the former. It was noticeable that many patterns of occurrence, very apparent at low levels, persisted, but less well defined, at higher levels of occurrence.

CHAPTER 6 – THE SPATIAL ARRANGEMENT, WITHIN A COLONY, OF THE AUTOZOOIDS AND HETEROZOOIDS OF *TRICELLARIA INOPINATA*

6.1 INTRODUCTION

The characteristics of the family Candidae were described in Section 5.1.1.

6.1.1 Characteristics of the genus *Tricellaria* Fleming, 1828

Colonies are erect, unilaminar, branching; one or both branches at bifurcations being jointed at their inception. They consist of two rows of autozooids staggered relative to one another, with a centrally placed distal autozooid proximal to each bifurcation. The arrangement of autozooids, relative to each other and to the joints at bifurcations, is of Type 9 or 11 of Harmer's 1923 classification (see Figure 6.1, below). Autozooids are narrow proximally and have an oval frontal membrane, usually with mural spines and a modified lateral spine, the scutum, which overarches it. The ancestrula is vase shaped with mural spines and the colony is attached to the substratum by tubular rhizoids. Distally sited lateral avicularia are found in most species, but not on all autozooids. There are no vibracula. Ovicells are sub-globular and hyperstomial. The genus differs from *Scrupocellaria* in the complete absence of vibracula and in the arrangement of autozooids at bifurcations: here the apical autozooid is more exposed.

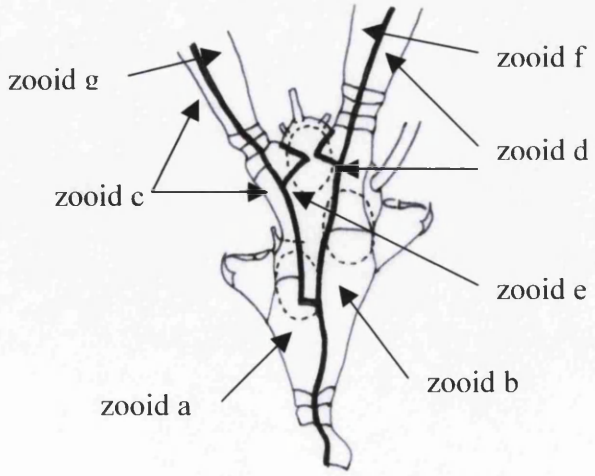


Figure 6.1 The arrangement of autozooids to each other, and to a bifurcation, in Harmer's bifurcation type 9 (modified after Hayward and Ryland)

6.1.2 General morphological description of *Tricellaria inopinata*

The morphological characteristics of *Tricellaria inopinata* d'Hondt and Occhipinti Ambrogi, 1985 were described in Section 3.3.1 in Chapter 3. The species is an arborescent cellularine bryozoan, with branches (internodes) which are unilaminar and biserial, and which bifurcate at intervals resulting in the characteristic bush-like form of the colony. *T. inopinata* is morphologically variable, in some respects so variable as to pose the question as to whether there is any obvious pattern to the occurrence of such variations, or correlations between them.

6.1.3 Objectives

The objectives of this study were the same as those described in Section 5.1.4, in respect of *Scrupocellaria reptans*; to describe the spatial arrangement of autozooids and heterozooids within a colony in as much detail as possible. Would this reveal any previously undescribed patterns, correlations or trends of zooid occurrence, and would any of these coincide with any recently revealed in respect of *S. reptans?* In respect of the autozooids, this could again provide direct information on the structure and form of a colony. Information on the spatial distribution of polymorphs was less likely to lead to definite conclusions and be more difficult to interpret.

The situation differed from the previous study in three respects:-

- Firstly, a spatial arrangement of autozooids within internodes, internodes within 'stem sequences', and 'stem sequences' within a colony, had been described for *S. reptans*, together with the structure and form of the colony. Superficially, *T. inopinata* appeared to be similar, in respect of the arrangement of autozooids within internodes, the nature of bifurcations, and the pattern of branching. Did *T. inopinata* have an identical or similar structure and form? If so, did that structure have a more widespread significance, occurring in at least two different genera? If it did not, perhaps a different colony structure would be revealed.

- Secondly, the situation for heterozooids was less complex than in *S. reptans,* in that there were fewer polymorphs. It was, therefore, conceivable that more progress could be made in trying to determine why their pattern of occurrence was as it was.
- Thirdly, all of the material came from one, recently discovered, population.

6.2 DETAILED INVESTIGATION INTO THE SPATIAL ARRANGEMENT OF AUTOZOOIDS AND HETEROZOOIDS WITHIN A SINGLE COLONY FROM POOLE HARBOUR

6.2.1 Introduction

Because the detailed study of *Scrupocellaria reptans,* described in Chapter 5, had essentially involved ~50% of a large colony, it was felt desirable here to study a complete, if somewhat smaller, colony.

6.2.2 Results

(The raw data are in Appendix J)

6.2.2.1 AUTOZOOIDS, INTERNODES AND 'STEM SEQUENCES'

6.2.2.1.1 The interrelationship of internodes at bifurcations

The situation here is identical to that obtaining in *S. reptans* and described in Section 5.3.2.1.1.

6.2.2.1.2 The numbers and lengths of internodes, complete and incomplete, and in relation to internode generation

How did the number of internodes, complete and incomplete, vary with internode generation?

Internode generation	Complete		Incomplete		Total	
	Frequency	%	Frequency	%	Frequency	%
15	0	0.0	3	1.6	3	0.6
14	2	0.7	12	6.2	14	2.8
13	15	4.9	17	8.8	32	6.4
12	29	9.5	25	13.0	54	10.9
11	41	13.5	38	19.7	79	15.9
10	50	16.4	33	17.1	83	16.7
9	44	14.5	25	13.0	69	13.9
8	37	12.2	27	14.0	64	12.9
7	33	10.9	11	5.7	44	8.9
6	23	7.6	1	0.5	24	4.8
5	15	4.9	1	0.5	16	3.2
4	8	2.6	0	0.0	8	1.6
3	4	1.3	0	0.0	4	0.8
2	2	0.7	0	0.0	2	0.4
1	1	0.3	0	0.0	1	0.2
Total	304	100.0	193	100.0	497	100.0

Table 6.1 The number and percentage occurrence of complete and incomplete internodes, by internode generation

Table 6.1 shows that exponential growth in terms of the number of internodes had ceased to occur by the sixth 'generation'. The maximum number of internodes, 83, occurred in the tenth 'generation', whilst the potential was for 512. Incomplete internodes, which were absent from the first four generations, steadily increased in number, and in the distalmost generations, outnumbered complete internodes.

For complete internodes was there any relationship between internode length and internode generation?

Inter-node gener-ation	Number of autozooids in internode					Number of internodes	Mean number of autozooids
	3	5	7	9	11		
	Frequency						
14		2				2	5.00
13	1	9	3	2		15	5.80
12	3	15	10	1		29	5.62
11	8	21	6	5	1	41	5.54
10	9	30	5	4	2	50	5.40
9	15	21	8			44	4.68
8	14	17	3	1	2	37	4.84
7	17	10	5	1		33	4.39
6	12	9	1		1	23	3.83
5	15					15	3.00
4	8					8	3.00
3	4					4	3.00
2	2					2	3.00
1	1					1	3.00
Total	109	134	41	14	6	304	4.86

Chi-Square 114.591 P= < 0.001 (49/90)

Table 6.2 Lengths of complete internodes, by internode generation

Table 6.2 shows that the shortest internodes, of three autozooids, dominated the more proximal generations of internodes. In this colony no longer internodes were found until the sixth generation. Beyond this, they became proportionally less numerous in each subsequent generation. Internodes of five and seven autozooids became proportionally more numerous and both increased and then decreased in number. Internodes of 9 and 11 internodes occurred sparsely throughout the central and more distal regions of the colony. The average length of internodes, beyond generation five, essentially increased generation on generation.

Which internode lengths occurred, and how frequently?

Autozooids in internode	Complete		Incomplete	
	Number	%	Number	%
1			12	6.2
2			25	13.0
3	109	35.9	20	10.4
4			28	14.5
5	134	44.0	29	15.0
6			20	10.4
7	41	13.5	16	8.3
8			16	8.3
9	14	4.6	7	3.6
10			7	3.6
11	6	2.0	6	3.1
12			3	1.6
13			1	0.5
14			2	1.0
15			1	0.5
Total	304	100.0	193	100.0

Table 6.3 The number and percentage occurrence of the various length internodes, both complete and incomplete

(The lengths of incomplete internodes are of much less value than those which are complete, although they are not valueless. Some 30% of incomplete internodes had seven or more autozooids and showed no signs of bifurcating).

From Table 6.3 it is apparent that:-

- All complete internodes were of an odd number of autozooids.
- Complete internodes ranged from 3 to 11 autozooids.
- 80% of complete internodes were of three or five autozooids.
- There were a small number of incomplete internodes which were as long, or longer, than the longest complete internode.
- Almost 40% of internodes were incomplete.

(As with *S. reptans* it was not possible to establish if incomplete internodes were still growing when the colony was collected).

6.2.2.1.3 'Stem' and 'branch' internodes

Were there equal numbers of 'stems' and 'branches, and was there a relationship between the length of a complete internode and whether it was a 'stem' or a 'branch'?

Autozooids in internode	'Stem' internodes		'Branch' internodes	
	Frequency	Percentage	Frequency	Percentage
3	43	39.8	65	60.2
5	115	85.2	20	14.8
7	20	48.8	21	51.2
9	4	28.6	10	71.4
11	1	16.7	5	83.3
Total	183	60.2	121	39.8

Chi-Square 14.329 P= < 0.001 ('stems' and 'branches')
Chi-Square 64.531 P= < 0.001 (2/20) ('stems' and 'branches' by length)

Table 6.4 The number and percentage occurrence of complete 'stem' and 'branch' internodes, by length

Three features are apparent from Table 6.4:-

- For complete internodes there were 50% more 'stems' than 'branches'.
- The breakdown between 'stem' and 'branch' internodes for each individual internode length was clearly not a simple 60/40 one, indeed not one approximated to this. Some 85% of internodes of five autozooids were 'stems' and similarly but conversely 60% of internodes of three autozooids were 'branches'.
- Internodes of more than five autozooids were more often 'branches' than 'stems' and this trend increased with internode length.

For incomplete internodes, how did the numbers of 'stems' and 'branches' compare?

Internode type	Frequency	Percentage occurrence
'stem'	75	39
'branch'	118	61

Chi-Square 9.580 P= 0.002

Table 6.5 The number and percentage occurrence of incomplete 'stem' and 'branch' internodes

Table 6.5 shows that for incomplete internodes, there were 50% more 'branches' than 'stems'.

How did the mean lengths of complete and incomplete 'stem' and 'branch' internodes compare?

Internode characteristics	Number of autozooids	Number of internodes	Mean number of autozooids
Complete 'stems'	899	183	4.86
Complete 'branches'	577	119	4.85
Incomplete 'stems'	378	75	5.04
Incomplete 'branches'	652	118	5.53

Table 6.6 The mean number of autozooids in complete and incomplete internodes, in 'stem' and 'branch' internodes

Table 6.6 shows that, on average, incomplete internodes were longer than complete, and that this was especially so in respect of 'branches'.

6.2.2.1.4 'Stem sequences'

6.2.2.1.4.1 The lengths of 'stem sequences'

How frequently did 'stem sequences' of different lengths occur (number of internodes, complete and incomplete) and how did their occurrence relate to their generation of origin?

Internodes in 'stem sequences'	Internode generation of origin															Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
15	1															1
14																
13																
12		1														1
11			1													1
10				1												1
9																
8				2			1									3
7				1												1
6			1		2	2	1			1						7
5						2	2	4	1							9
4							2	3	1	2						8
3					1	5	3	3	6	3	5	1				27
2					1	2	3	4	6	8	8	4	2			38
1					4	1	10	19	19	26	25	18	11	8	1	142
Total	1	1	2	4	8	12	22	33	33	40	38	23	13	8	1	239

Table 6.7 Occurrence, in terms of 'stem sequence' lengths (number of complete and incomplete internodes) of all 'stem sequences', by their generation of origin

Table 6.7 shows that there were a small number of long 'stem sequences', originating in the first few generations of internodes, and a much larger number of short or very short 'stem sequences'. Over 93% of 'stem sequences' were of five or fewer internodes, and less than 2% were of 11 or more.

6.2.2.1.4.2 The relationship between the length of complete internodes and their position within their 'stem sequence'

(The data relating to the lengths of all of the complete internodes and their sequence, within the 'stem sequences' initiated in the first 10 generations of internodes, is in Appendix 'K').

Was there a relationship between the length of a complete internode and its numerical position within its 'stem sequence'?

Numerical position of internode within 'stem sequence'	Length of internode				
	3	5	7	9	11
14		1			
13		1			
12	1	1			
11		3			
10	2	1			
9	1	3			
8	2	2	1		
7	2	5	1		
6	3	7			
5	4	12	1		
4	7	18	1	1	
3	11	21	3	2	
2	10	40	13	1	1
1	65	20	21	10	5
Total	108	135	41	14	6

Table 6.8 Numbers of the various lengths of complete internodes by their numerical position within their 'stem sequence'

Table 6.8 shows that, generally, only internodes of five and to a lesser extent three autozooids occurred within the more distal internode positions within a 'stem sequence'. The longer an internode, beyond those of five autozooids, the more pronounced their concentration in the more proximal positions.

6.2.2.1.4.3 Internode lengths and their sequence within the four longest 'stem sequences'

Did these long 'stem sequences' exhibit any particular characteristics in terms of the lengths, and/or sequence, of their constituent autozooids?

Internode generation	Internode lengths and sequence			
14	5			
13	5	5	5	
12	3	5	5	5
11	5	3	3	5
10	3	5	5	3
9	5	3	3	5
8	3	5	5	5
7	5	3	3	3
6	3	3	5	5
5	3	3	3	3
4	3	3	3	3
3	3	3	3	
2	3	3		
1	3			
Internode of Origin	A1	B2	C4	D4

Table 6.9 Internode lengths, and their sequence, within the four longest 'stem sequences'

Table 6.9 shows that:-

- No internodes longer than five autozooids were present.
- All of the internodes of the first five generations were of three autozooids.
- There was a marked tendency, beyond the first five generations, for internodes of three and five autozooids to occur alternately.

How did the internode composition of the four longest 'stem sequences' compare with that of the colony overall?

Autozooids in Internode	Complete colony		Four longest 'stem sequences'	
	Number	Percentage occurrence	Number	Percentage occurrence
3	109	36	27	59
5	134	44	19	41
7	41	13	0	0
9	14	5	0	0
11	6	2	0	0
Total	304	100	46	100

Chi-Square 16.003 P= < 0.01

Table 6.10 The number and percentage occurrence of complete internodes of different lengths, within the colony, and in the four longest 'stem sequences'

Table 6.10 shows that:-

- Within the four longest 'stem sequences', internodes of three autozooids occurred much more frequently than in the colony overall.
- Internodes of 7, 9 and 11, 20% of internodes within the colony, were completely absent from the four longest 'stem sequences'.

As discussed in Chapter 5 (Section 5.3.2.1.4.3) in respect of *S. reptans*, the internode composition of the complete colony, and the long 'stem sequences, here four, also differed in two important respects:-

- 1) The proportions of the different length internodes in the various generations of internodes.
- 2) The proportions of 'stem' and 'branch' internodes.

Could these differences account for the differences observed in the internode composition of the long 'stem sequences' and the colony?

With regard to 1) above:-

Did differences in the proportions of the different length internodes per generation within the colony, and within the four longest 'stem sequences', account for the differences in the internode composition of the two?

Inter-node gener-tion	Number of autozooids in internode									
	3		5		7		9		11	
	Frequency									
	Act	Exp	Act	Exp	Act	Exp	Act	Exp	Act	Exp
14			1	1.0						
13	0	0.2	3	1.8	0	0.6	0	0.4		
12	1	0.4	3	2.1	0	1.4	0	0.1		
11	2	0.8	2	2.0	0	0.6	0	0.5	0	0.1
10	2	0.7	2	2.4	0	0.4	0	0.3	0	0.2
9	2	1.4	2	1.9	0	0.7				
8	1	1.5	3	1.8	0	0.3	0	0.1	0	0.2
7	3	2.1	1	1.2	0	0.6	0	0.1		
6	2	2.1	2	1.6	0	0.2			0	0.2
5	4	4.0								
4	4	4.0								
3	3	3.0								
2	2	2.0								
1	1	1.0								
Total	27	23.2	19	15.8	0	4.8	0	1.5	0	0.7

Table 6.11 Comparison of the actual numbers of the various lengths of complete internodes in the four longest 'stem sequences', with the numbers expected, if the proportions of internodes in the various generations were the same as in the colony

Table 6.11 shows that if the proportions of the various internode lengths in the four longest 'stem sequences' were the same as in the colony as a whole, one would have expected slightly fewer internodes of three and five autozooids. A minority of longer internodes would also have been expected.

If the figures in Figure 6.11 are added to those of 6.10, did they account for the differences within that table?

Autozooids in internode	Complete colony		Four longest 'stem sequences'			
			Actual internode composition		Expected internode composition	
	Number	%	Number	%	Number	%
3	109	36	27	59	23.2	50.4
5	134	44	19	41	15.8	34.4
7	41	13	0	0	4.8	10.4
9	14	5	0	0	1.5	3.3
11	6	2	0	0	0.7	1.5
Total	304	100	46	100	46.0	100.0

Chi-Square, 8.270 P= < 0.1

Table 6.12 The figures derived from Table 6.11 are added to the original comparison between the internode composition of the colony and the four longest 'stem sequences' (Table 6.10)

Table 6.12 appears to show that this factor, the difference between the proportions of the different lengths of internodes within the different internode generations within the colony and in the four longest 'stem sequences', could explain some, but not all, of the observed differences in respect of internodes of three and five autozooids. The absence of any internodes of seven or more autozooids from the four longest 'stem sequences' cannot however be explained thus; some 15% of such internodes could have been expected. The figures are not statistically significant, I believe, because the colony was not sufficiently well developed. The proximal generations, of short internodes are therefore over represented, and the distal generations, in which long internodes occur more frequently, under represented.

With regard to 2) above:-

Autozooids in internode	Complete colony		Four longest 'stem sequences'			
			Actual internode composition		Expected internode composition	
	Number	%	Number	%	Number	%
3	109	36	27	59	16.5	36
5	134	44	19	41	20.4	44
7	41	13	0	0	6.1	13
9	14	5	0	0	2.1	5
11	6	2	0	0	0.9	2
Total	304	100	46	100	46.0	100

Chi-Square 15.878 P= < 0.01

Table 6.13 The figures derived from calculating the expected numbers of different length internodes as if the proportion of 'stems' to 'branches' was the same as that in the colony as a whole; related to those comparing the numbers of such internodes in the colony, and the four longest 'stem sequences' (Table 6.11)

Table 6.13 shows that the imbalance between the number of 'stem' and 'branch' internodes of the various length complete internodes within the colony, and the very different numbers in the four longest 'stem sequences', does not account for the differences observed between them. Internodes of three autozooids occurred much more frequently than would be expected, and internodes of seven, nine and eleven were absent altogether, although on this basis, one would have expected them to constitute some 20% of the total.

How did the mean number of autozooids per internode within the colony, compare with that in the four longest 'stem sequences'?

	Number of autozooids	Number of internodes	Mean number of autozooids
Complete colony	1476	304	4.85
4 longest 'stem sequences'	176	46	3.83

Chi-square, Yates' Correction for Continuity 9.733 $P = < 0.01$

Table 6.14 The mean lengths of complete internodes within the colony and in the four longest 'stem sequences'.

Table 6.14 shows that, on average, complete internodes of the colony were one autozooid longer, than those of the four longest 'stem sequences'.

6.2.2.1.4.4 The spatial arrangement of internodes within the colony, particularly in relation to the four longest 'stem sequences'

(The spatial arrangement of all the internodes within the first eight generations, together with that of ovicells, is shown in Figure 6.2).

- The first autozooid of 'stem' internodes are shaded.
- Incomplete internodes are indicated by a dot following the most distal autozooid.
- Ovicells are shown in red.

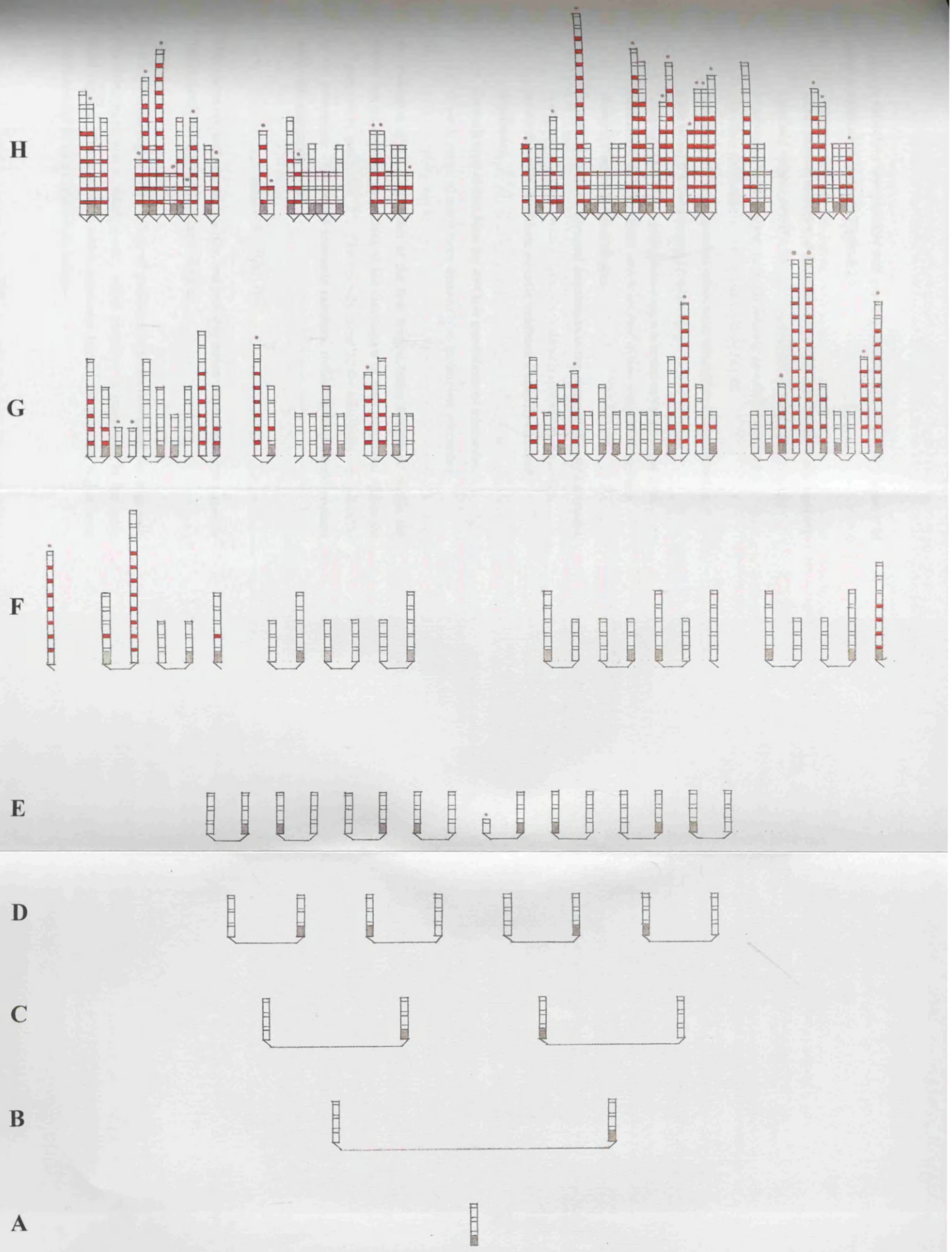


Figure 6.2 The spatial arrangement of all of the internodes within the first eight generations of internodes, showing internode lengths and ovicell occurrence

Although the colony was probably still growing when it was collected, a number of characteristics are apparent in Figure 6.2:-

- The consistent character of the internodes of the first five generations, being short and without ovicells; and their persistence, although in decreasing numbers in the subsequent three generations, in which long, largely ovicellate internodes became numerically dominant.
- A majority of the longest internodes were incomplete (long internodes often brought a 'stem sequence' to an end).
- Except where the internodes following a bifurcation were both of three autozooids, all bifurcations which resulted in two complete internodes, produced two of different lengths.
- Ovicells exhibited a clumped distribution in two respects. Firstly internodes were generally either without ovicells or densely ovicellate. Secondly, internodes with, or without ovicells, exhibited a laterally clumped distribution.
- Ovicells were absent from the first five generations of internodes.
- Ovicells occurred much more frequently in incomplete internodes.

An indication of the distribution of the four longest 'stem sequences' within the colony can be obtained by looking at the distribution of their internodes within the 12th generation, generation 'L'. Theoretically some 2000+ internodes were possible in this generation. The actual internode numbers, indicating their lateral position within this generation were:-

683; 854; 1366; 1707.

These were not well spaced, laterally, and probably reflect either damage or a growth pattern much influenced by spatial constraints.

In the proximal region of the colony all possible internodes developed, but as growth proceeded there was a 'thinning-out', which increased in magnitude in the more distal regions. The manner in which internodes beyond the proximal region were distributed can be seen in the details below:-

In the 12th generation of internodes, generation 'L', some 2000+ internodes were theoretically possible. In this colony some 54 internodes had developed in this generation and their clumped distribution is evident from the following:-

3 internodes occurred between positions 330 and 332 (all were incomplete)
* 18 internodes occurred between positions 651 and 696
* 6 internodes occurred between positions 854 and 860
* 13 internodes occurred between positions 1361 and 1387
1 internode occurred in position 1454 (incomplete)
* 9 internodes occurred between positions 1705 and 1718
4 internodes occurred between positions 1877 and 1881

* Each of the four larger clusters had an internode of one of the four longest 'stem sequences' within it.

[The pattern was very similar to that found in *S. reptans,* but the colony here was not as well developed].

Figure 6.3 shows, for a number of generations, the proportion of available internode positions, which were actually 'occupied', and how these were distributed in each generation.

- The dashed vertical lines indicate the long 'stem sequences'.
- The dotted vertical line a 'stem sequence' which was not very long but which had the internode composition of such a 'stem sequence' (possibly damaged).
- The boxes and vertical lines within each generation the lateral extent of internode occurrence.

The figure inevitably gives a very false impression of the numbers of internodes occurring in each generation.

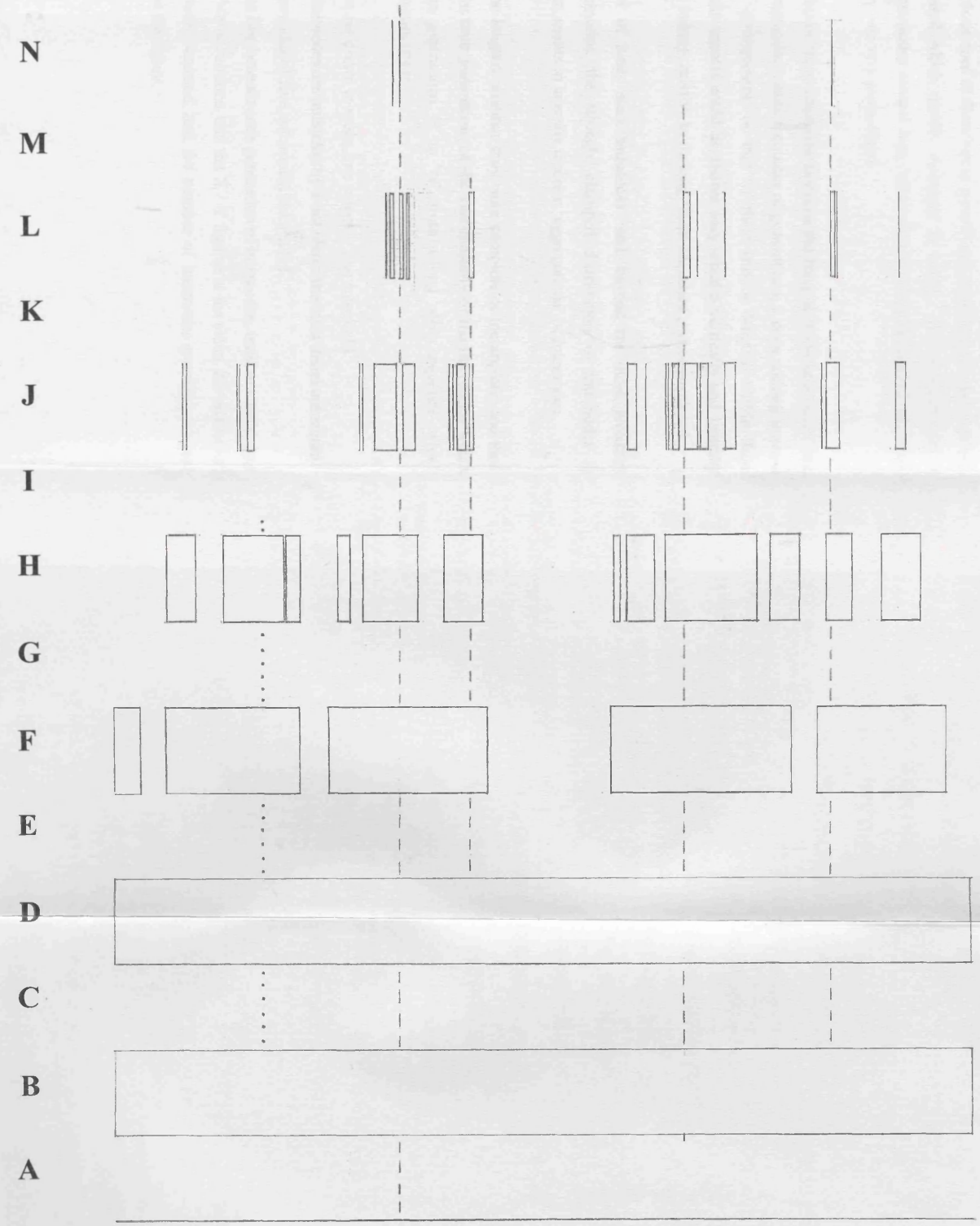


Figure 6.3 The positions of internodes produced, relative to potential but unoccupied 'sites', in relation to long 'stem sequences'.

Figure 6.3 shows the relationship, within a number of generations, between the long 'stem sequences' and the distribution of internodes. Initially internodes were produced in all positions open to them but as growth continued, gaps between groups of internodes appeared which rapidly increased in width. The concentration of narrow groups of internodes around long 'stem sequences' in the central and distal regions of the colony was very pronounced.

Whilst Figure 6.3 shows the relationship between the longest 'stem sequences' and the distribution of internodes within a number of generations, it does nothing to show their actual spatial arrangement, or that of their various lengths, within these 'aggregations'. These aspects could be shown only when a vertically and laterally limited region of the colony, relative to its long 'stem sequence', was considered.

The limited number of long 'stem sequences' and, beyond the more proximal generations of internodes, the strongly clumped distribution of internodes in association with them results in laterally discrete 'aggregations' of internodes.

Figure 6.4, shows the lengths, whether they were complete or incomplete, and their actual position within their generation, of the vast majority of the internodes which were generated, from generations 'F' to 'N', from a long 'stem sequence' which developed from internode 'F11'.

- All internodes are drawn to scale, one square = one autozooid.
- Incomplete internodes are indicated by a dot above the most distal autozooid.
- Complete internodes of five autozooids are shaded.
- The horizontal line beneath each generation of internodes, indicates the extent of their possible occurrence, and the X / Y figures at the sides, the number of internodes which occurred, and the number of internodes possible, beyond the margins of the figure.

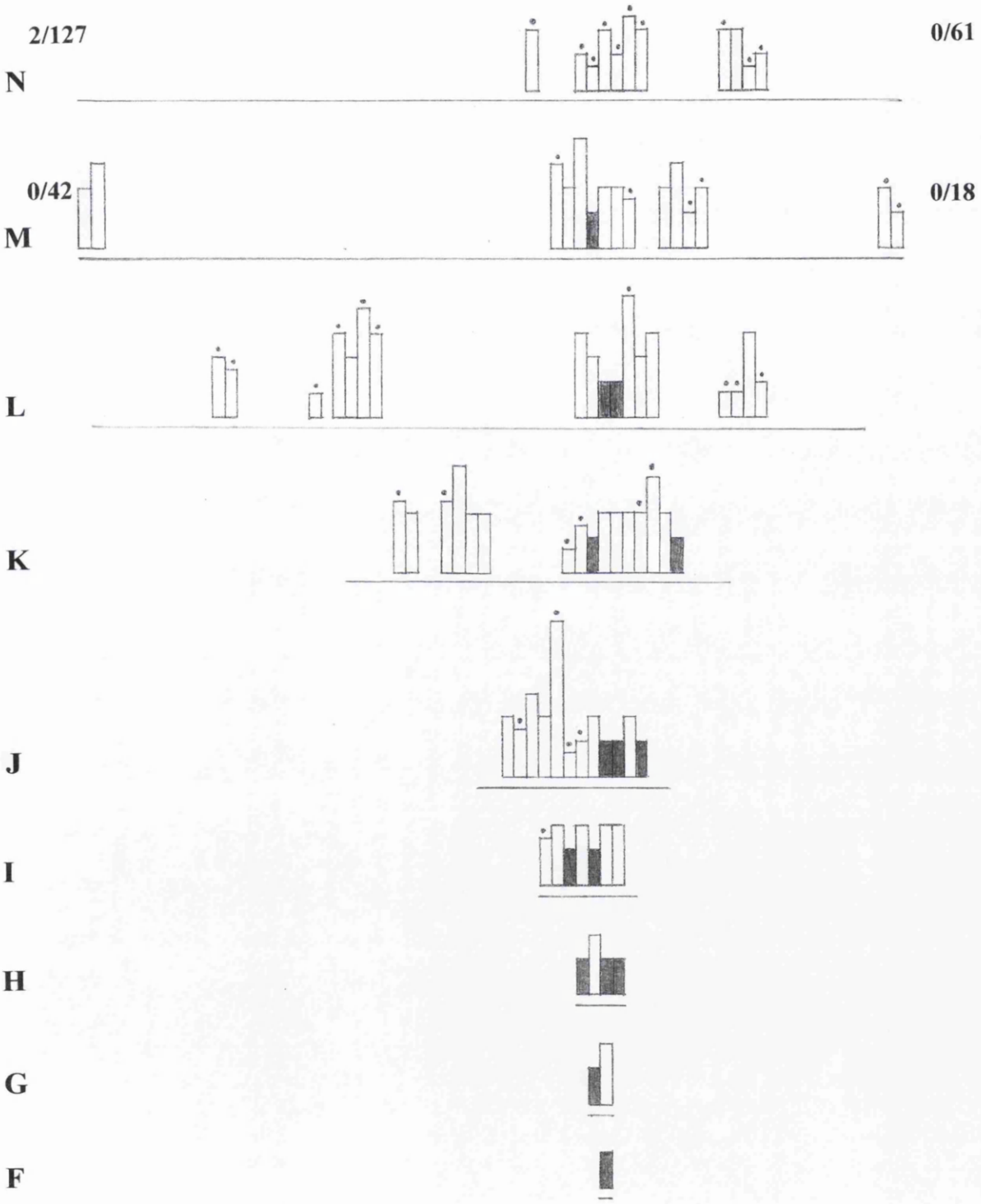


Figure 6.4 The pattern of internode presence and absence (and their length) for all of the internodes which could have developed from a long 'stem sequence' which developed from internode 'F11'.

From Figure 6.4 it is clear that internodes were very strongly concentrated around the long 'stem sequence'. Although this colony was not as well developed as that of *S. reptans,* considered in Chapter 5, the pattern of internode occurrence was very similar. An 'aggregation', in two dimensions, was essentially a truncated lanceolate shape, and there was also some evidence, of the development of the occasional lateral 'arm' outside the central band, found in that colony.

The two-dimensional lanceolate shape of an 'aggregation' of internodes, developing from a long 'stem sequence', becomes fusiform in three dimensions. The pronounced angulation between internodes at bifurcations, and to a lesser degree, the lengthwise concavity of the longer internodes, results in incurving, both distally and laterally. As a result 'aggregations' often form semi-clenched fist-like structures. The overall three-dimensional structure of the colony is very dense. It is, essentially an incomplete circle of these structures which develop from a vertically limited, laterally continuous, proximal region.

6.2.2.2 POLYMORPH OCCURRENCE AND DISTRIBUTION

6.2.2.2.1 Lateral avicularia

The preliminary investigation of a proximal region of a colony led me to believe that lateral avicularia occurred frequently, perhaps on 50% of the autozooids which could give rise to them.

On closer examination of this colony, it became apparent that the level of lateral avicularia occurrence, taking the colony as a whole, was much lower. Further, their distribution exhibited no simple pattern. (Lateral avicularia in this species vary in size, but this aspect will be considered later).

Was lateral avicularia occurrence related to the internode generation, in which they occurred?

Internode generation	Lateral avicularia		Percentage occurrence
	Present	Absent	
13	1	2	33.3
12	12	48	20.0
11	42	190	18.1
10	68	255	21.0
9	70	232	23.1
8	80	214	27.2
7	56	113	33.1
6	50	26	65.7
5	17	12	58.6
4	13	3	81.2
3	5	3	62.5
2	2	0	100.0
1	0	0	0.0
Total	416	1098	27.4

Chi-Square 127.698 P= < 0.001 (6/25)

Table 6.15 The number and percentage occurrence of lateral avicularia by internode generation (apical autozooids and 'x' and 'i' cases excluded)

Table 6.15 shows that, whilst lateral avicularia occurrence was initially high in proximal generations, it declined suddenly and remained low thereafter.

Was lateral avicularia occurrence related to the length of the internode in which they occurred?

Autozooids in internode	Lateral avicularia		Percentage occurrence
	Present	Absent	
11	16	40	28.6
9	22	61	26.5
7	62	111	35.8
5	174	226	43.5
3	79	117	40.3
Total	353	555	38.9

Chi-square 12.287 P= 0.015

Table 6.16 Lateral avicularia presence or absence by length of complete internodes (apical autozooids and 'x' and 'i' cases excluded)

Table 6.16 shows no evidence, that the occurrence of lateral avicularia was related to internode length in any consistent way.

Was lateral avicularia occurrence related to whether the internode in which it occurred was complete or incomplete?

Internode type	Lateral avicularia		Percentage occurrence
	Present	Absent	
Complete	353	555	38.8
Incomplete	63	543	10.3
Total	416	1098	27.4

Chi-Square, Yates' Correction for Continuity 146.515 P= < 0.001

Table 6.17 Lateral avicularia presence or absence by complete and incomplete internodes (apical autozooids and 'x' and 'i' cases excluded.)

Table 6.17 shows that lateral avicularia occurred almost four times more frequently in complete than incomplete internodes.

Was lateral avicularia occurrence related to whether an internode was a 'stem' or a 'branch'?

Internode type	Lateral avicularia		Percentage occurrence
	Present	Absent	
'stem'	269	475	36.1
'branch'	147	623	19.0

Chi-Square, Yates' Correction for Continuity 54.445 P= < 0.001

Table 6.18 Lateral avicularia presence or absence by 'stem' and 'branch' internodes (apical autozooids and 'x' and 'i' cases excluded.)

Table 6.18 shows that lateral avicularia occurred almost twice as frequently in 'stem' than 'branch' internodes.

Was lateral avicularia occurrence related to autozooid position within an internode?

Autozooid position	Lateral avicularia		Percentage occurrence
	Present	Absent	
Sub-apical	289	189	60.4
Proximal	93	795	10.4
Total	382	984	27.9

Chi-Square, Yates' Correction for Continuity 382.958 P= < 0.001

Table 6.19 Lateral avicularia presence or absence by autozooid position within an internode (apical autozooids and 'x' and 'i' cases excluded.)

Table 6.19 shows that lateral avicularia occurred ~6 times more frequently on sub-apical than proximal autozooids.

Was the frequency of lateral avicularia occurrence related to autozooid number?

Autozooid number	Lateral avicularia		Percentage occurrence
	Present	Absent	
13	1	1	50.0
12	1	3	25.0
11	3	3	50.0
10	2	9	18.1
9	10	10	50.0
8	8	30	21.0
7	22	24	47.8
6	25	64	28.0
5	44	63	41.1
4	85	145	36.9
3	101	139	42.0
2	79	278	22.1
1	35	329	9.6
Total	416	1098	27.4

Chi-Square 127.445 P= < 0.001 (7/27)

Table 6.20 Lateral avicularia presence or absence by autozooid number (apical autozooids and 'x' and 'i' cases excluded.)

From Table 6.20 a definite, if complex pattern, was apparent. For the most proximal pair of autozooids, lateral avicularia occurred infrequently but were twice as numerous on the even- numbered of the pair. Thereafter they occurred more frequently on the odd-numbered; increasingly so with increasing autozooid number.

Was lateral avicularia occurrence related to odd and even-numbered autozooids?

Autozooid number	Lateral avicularia		Percentage occurrence
	Present	Absent	
Odd-numbered	216	569	27.5
Even-numbered	200	529	27.4
Total	416	1098	27.5

Chi-Square, Yates' Correction for Continuity .000 P= 1.000

Table 6.21 Lateral avicularia occurrence by odd and even-numbered autozooids (apical autozooids and 'x' and 'i' cases excluded)

Table 6.21 shows that, overall, lateral avicularia occurred with equal frequency on odd and even-numbered autozooids.

If lateral avicularia occurrence is considered relative to both autozooid position, within an internode, and internode generation, was any pattern apparent?

Internode generation	Lateral avicularia					
	Proximal autozooids			Sub-apical autozooids		
	Present	Absent	%	Present	Absent	%
13		2	0.0			
12	2	38	5.0	8	3	72.7
11	7	139	4.8	29	30	49.2
10	6	186	3.1	56	40	58.3
9	17	175	8.8	48	35	57.8
8	24	164	12.8	47	24	66.2
7	16	80	16.7	36	25	59.0
6	21	11	65.6	28	14	66.6
5				17	12	58.6
4				13	3	81.3
3				5	3	62.5
2				2	0	100.0
1						
Total	93	795	10.5	289	189	60.5

Table 6.22 Lateral avicularia presence or absence by both internode generation and autozooid position within an internode (apical autozooids and 'x' and 'i' cases excluded.)

Table 6.22 shows that the percentage of sub-apical autozooids which gave rise to lateral avicularia, remained high and constant in all generations. The situation was very different in respect of proximal autozooids. Because the first five generations

of internodes were all of three autozooids, there were no proximal autozooids here; but they were numerous from the sixth generation on. Lateral avicularia occurred on 66% of such autozooids in generation six, but thereafter declined rapidly and remained at a very low level in all subsequent generations. The internode generation factor operated only on proximal autozooids. Because it was known that lateral avicularia were negatively correlated with ovicells and related to internode generation, it seemed worthwhile to calculate the figures for the parameters included in Table 6.22 for just the four longest 'stem sequences'. The relationship here, between internode generation and ovicell occurrence, was very different from that in the colony as a whole; although the numbers were, of course, very small.

If lateral avicularia occurrence was considered relative to both autozooid position, within an internode, and internode generation, within the four longest 'stem sequences', was the pattern as that in Table 6.22?

Internode Generation	Lateral avicularia					
	Proximal autozooids			Sub-apical autozooids		
	Present	Absent	%	Present	Absent	%
13						
12						
11	1	1	50	3	3	50
10	0	4	0	7	1	87
9	3	1	75	4	4	50
8	3	2	60	7	1	87
7	1	1	50	5	3	62
6	2	0	100	4	3	57
5				5	3	62
4				8	0	100
3				4	2	66
2				2	0	100
1						
Total	10	9	52	49	20	71

Table 6.23 Lateral avicularia presence or absence by internode generation and autozooid position within an internode in the four longest 'stem sequences' (apical autozooids and 'i' and 'x' cases excluded.)

Table 6.23 shows, although the figures are very small, that the percentage of autozooids producing lateral avicularia in the four longest 'stem sequences was 67% (whilst within the colony it was only 27.5%). The sudden decline in lateral

avicularia occurrence from internode generation seven on, a notable feature in Table 6.22, is not apparent in Table 6.23.

Lateral avicularia occur in a variety of sizes, from very small to very large (and to a lesser degree morphologies) and it is worth noting that the largest forms occurred only on the distalmost autozooid in the external series of autozooids in an internode.

Was lateral avicularia presence or absence related to the length of the 'stem sequence' in which they occurred?

No. of internodes in 'stem sequence'	Lateral avicularia		Percentage occurrence
	Present	Absent	
15	18	6	75.0
12	15	9	62.5
11	14	9	60.9
10	13	6	68.4
8	32	29	52.5
7	15	11	57.7
6	45	58	43.7
5	44	86	33.8
4	26	57	31.3
3	73	179	29.0
2	52	209	19.9
1	69	439	13.6
Total	416	1098	27.5

Chi-Square 175.663 P= < 0.001

Table 6.24 Lateral avicularia presence or absence by length of 'stem sequence' in which they occurred (apical autozooids and 'x' and 'i' cases excluded).

Table 6.24 shows that the longer the length of a 'stem sequence' the higher the level of lateral avicularia occurrence.

Was lateral avicularia presence or absence related to the numerical position of an internode within a 'stem sequence'?

Numerical position of internode within 'stem sequence'	Lateral avicularia		Percentage occurrence
	Present	Absent	
11	3	1	75.0
10	3	1	75.0
9	5	5	50.0
8	6	8	42.9
7	13	12	52.0
6	17	25	40.5
5	18	32	36.0
4	36	53	40.4
3	59	119	33.1
2	105	217	32.6
1	151	625	19.5
Total	416	1098	27.5

Chi-Square 65.882 P= < 0.001 (6/27)

Table 6.25 Lateral avicularia occurrence by numerical position of internode within its 'stem sequence' (apical autozooids and 'x' and 'i' cases excluded)

Table 6.25 shows that the incidence of lateral avicularia increased in relation to the numerical position of an internode within its 'stem sequence'. Investigation of 'stem sequences of equal length established that this did not occur within a 'stem sequence' but resulted from the higher level of occurrence in longer 'stem sequences', shown in Table 6.24.

Was there any relationship between the occurrence of lateral avicularia and the presence of ovicells?

Ovicell	Lateral avicularia		Percentage occurrence
	Present	Absent	
Present	81	791	9.2
Absent	285	236	54.7

Chi-Square, Yates' Correction for Continuity 344.895 P= < 0.001

Table 6.26 Lateral avicularia presence or absence related to the presence or absence of ovicells (apical autozooids and 'x' and 'i' cases excluded)

Table 6.26 shows that lateral avicularia occurred ~6 times more frequently on non-ovicellate autozooids (the autozooid producing the embryo not the ovicell).

6.2.2.2.2 Rhizoids

Rhizoids were heavily concentrated within the more proximal region of the colony, and laterally within long 'stem sequences', and internodes adjacent to them.

6.2.2.3 THE DISTRIBUTION OF OVICELLS THROUGHOUT THE COLONY

In this section, ovicells were used as indicators of the distribution of autozooids producing embryos requiring ovicells; i.e. the autozooid referred to was the one proximal to that actually producing the ovicell. The opening to the ovicell is however adjacent to any lateral avicularium produced.

Was ovicell occurrence related to internode generation?

Internode Generation	Ovicells		Percentage Occurrence
	Present	Absent	
15	0	2	0
14	14	6	70
13	63	40	61
12	131	70	65
11	184	92	67
10	206	103	67
9	199	93	68
8	196	87	69
7	104	63	62
6	21	61	26
5	0	31	0
4	0	16	0
3	0	8	0
2	0	4	0
1	0	2	0
Total	1118	678	62

Chi-Square 167.124 P= < 0.001 (8/27)

Table 6.27 The number and percentage occurrence of ovicells by internode generation (apical autozooids and 'x' and 'i' cases excluded)

Table 6.27 shows that in the colony as a whole ovicells occurred on >60% of autozooids able to produce them, and that they were completely absent from the first five generations. The sixth generation appeared to be a transitional one, with some 26% of autozooids producing ovicells. Thereafter ovicell production was consistently >60%.

Was ovicell production related to the lengths of complete internodes in which they occurred?

Autozooids in Internode	Ovicell		Percentage occurrence
	Present	Absent	
11	47	8	85
9	77	20	79
7	148	86	63
5	189	324	37
3	7	207	3

Chi-Square 278.925 P= < 0.001

Table 6.28 The number and percentage occurrence of ovicells by length of complete internodes (apical autozooid and 'x' and 'i' cases excluded)

Table 6.28 shows that the longer the internode, the greater the percentage of autozooids which gave rise to ovicells.

Was ovicell production related to whether an internode was a 'stem' or a 'branch'?

Internode type	Ovicell		Percentage occurrence
	Present	Absent	
'stem'	455	443	50.7
'branch'	663	235	73.8

Chi-Square, Yates' Correction for Continuity 101.526 P= < 0.001

Table 6.29 Ovicell presence or absence by 'stem' and 'branch' internodes (apical autozooids and 'x' and 'i' cases excluded)

Table 6.29 shows that ovicells occurred ~50% more frequently on autozooids in 'branch' than 'stem' internodes.

Was ovicell production related to the autozooid number and/or position within an internode?

Autozooid number	Proximal autozooid position			Sub-apical autozooid position		
	Autozooids	Ovicells	%	Autozooids	Ovicells	%
12	3	3	100	0	0	0
11	3	3	100	0	0	0
10	6	6	100	4	0	0
9	7	7	100	4	0	0
8	24	24	100	9	0	0
7	27	27	100	9	0	0
6	60	58	97	36	3	8
5	60	57	95	38	2	5
4	132	124	94	129	18	14
3	140	136	97	126	8	6
2	317	267	84	107	7	7
1	319	255	80	107	0	0
Total.	1098	967	88	569	38	7

Chi-Square 1032.321 P= < 0.001 (Proximal/Sub-apical)

Table 6.30 The number and percentage occurrence of ovicells by autozooid number and autozooid position within an internode (apical autozooids and 'x' and 'i' cases excluded.)

Table 6.30 shows that:-

- Ovicell occurrence was not related to autozooid number.
- Whilst 88% of all 'proximal' autozooids were ovicellate, 93% of sub-apical autozooids were not.

Was the occurrence of ovicells related to odd and even-numbered autozooids?

Autozooid number	Ovicell		Percentage occurrence
	Present	Absent	
Odd-numbered	576	357	61.7
Even-numbered	542	321	62.8
Total	1118	678	62.2

Chi-Square, Yates' Correction for Continuity .174 P= 0.676

Table 6.31 Ovicell occurrence by odd and even-numbered autozooids (apical autozooids and 'x' and 'i' cases excluded)

Table 6.31 shows no evidence that ovicell occurrence was related to odd and even-numbered autozooids.

If ovicell occurrence is considered relative to both autozooid position within an internode, and internode generation, was any pattern apparent?

Internode Generation	Ovicell					
	Proximal autozooids			Sub-apical autozooids		
	Present	Absent	%	Present	Absent	%
15	0	2	0			
14	10	2	83	0	4	0
13	51	12	81	0	28	0
12	114	16	88	2	54	4
11	160	20	89	9	62	13
10	172	22	89	11	81	12
9	176	16	92	9	76	11
8	172	18	91	4	66	6
7	92	7	93	3	56	5
6	20	16	56	0	44	0
5				0	30	0
4				0	16	0
3				0	8	0
2				0	4	0
1				0	2	0
Total	967	131	88	38	531	7

Table 6.32 Ovicell presence or absence by internode generation and autozooid position within an internode (apical autozooids and 'x' and 'i' cases excluded)

Table 6.32 shows that for 'proximally' sited autozooids, once ovicells were produced they continued to be so in each generation at a very high rate. It also shows that sub-apical autozooids rarely produced ovicells, and that ovicell production by such autozooids commenced later, and was concentrated in a small number of internode generations.

What is not apparent from the data is the extent to which ovicells were concentrated within distally sited, long internodes, which were often incomplete.

Was ovicell production related to whether an internode was complete or incomplete?

Internode type	Ovicells		Percentage occurrence
	Present	Absent	
Complete	468	645	42
Incomplete	650	33	95
Total	1118	678	62

Chi-Square, Yates' Correction for Continuity 505.977 P= < 0.001

Table 6.33 The number and percentage occurrence of ovicells by complete and incomplete internodes (apical autozooids and 'x' and 'i' cases excluded)

Table 6.33 shows that ovicells occurred more than twice as frequently in incomplete than complete internodes.

Was ovicell presence or absence related to the length of the 'stem sequence' in which they occurred?

No. of internodes in 'stem sequence'	Ovicell		Percentage occurrence
	Present	Absent	
15	1	39	2.5
12	0	33	0.0
11	0	32	0.0
10	0	28	0.0
8	17	54	23.9
7	8	18	30.8
6	37	87	29.8
5	55	85	39.3
4	45	56	44.6
3	190	103	64.8
2	228	77	74.8
1	537	66	89.1
Total	1118	678	62.2

Chi-Square 575.231 P= < 0.001

Table 6.34 Ovicell occurrence by the length of the 'stem sequence' in which they occurred (apical autozooids and 'x' and 'i' cases excluded)

Table 6.34 shows that the level of ovicell occurrence was inversely related to 'stem sequence' length; being heavily concentrated in short 'stem sequences'.

How did the frequency of ovicell occurrence, within the four longest 'stem sequences', compare with that in all other 'stem sequences'?

	Ovicells		Percentage occurrence
	Present	Absent	
4 longest 'stem sequences'	1	129	0.8
All other 'stem sequences'	1117	546	67.2

Table 6.35 The number and percentage occurrence of ovicells within the four longest 'stem sequences' and in all other internodes (apical autozooids and 'x' and 'i' cases excluded)

Table 6.35 shows that whilst ovicells were abundant within the colony they were virtually absent from the four longest 'stem sequences'.

Was ovicell presence or absence related to the numerical position of an internode within a 'stem sequence'?

Numerical position of internode within 'stem sequence'	Ovicell		Percentage occurrence
	Present	Absent	
15	0	2	0.0
14	0	4	0.0
13	1	3	25.0
12	0	6	0.0
11	0	12	0.0
10	0	8	0.0
9	0	14	0.0
8	4	15	21.1
7	4	26	13.3
6	17	25	40.5
5	24	47	33.8
4	43	62	41.0
3	127	81	61.1
2	232	135	63.2
1	666	238	73.7
Total	1118	678	62.2

Chi-Square 226.140 P= < 0.001 (11/37)

Table 6.36 Ovicell occurrence by numerical position of an internode within its 'stem sequence' (apical autozooids and 'x' and 'i' cases excluded).

Table 6.36 shows that the level of ovicell occurrence decreased in relation to increased numerical position of an internode within its 'stem sequence'.

Investigation of 'stem sequences' of equal length established that this did not occur within a 'stem sequence', but resulted from the higher level of occurrence in shorter 'stem sequences', shown in Table 6.34.

It was apparent in the section on lateral avicularia that they were negatively correlated with the presence of ovicells, and Table 6.37 rearranges this data in respect of ovicells.

Lateral avicularia	Ovicells		Percentage occurrence
	Present	Absent	
Present	81	285	22
Absent	791	236	77

Chi-Square, Yates' Correction for Continuity 344.895 P= < 0.001

Table 6.37 Ovicell presence or absence related to the presence or absence of lateral avicularia (apical autozooids and 'x' and 'i' cases excluded)

Table 6.37 shows that ovicells occurred 3.5 times more frequently on autozooids without lateral avicularia.

6.3 SUPPLEMENTARY STUDIES

As discussed in Section 4.5.4.2, the colony investigated in detail in Section 6.2 was a single, not very large colony. To address this, at least in part, it was felt desirable to carry out a number of supplementary studies, concentrating on aspects where such were particularly necessary.

6.3.1 'Stem sequences' and internode lengths

The colony of the detailed investigation was not fully developed, and the information regarding the occurrence of various length 'stem sequences' was therefore not as conclusive as in the colony of *S. reptans.* A substantial portion of a large colony was investigated, solely in respect of all of its 'stem sequences' and their internode composition. The investigation did not utilize the recording scheme. 'Stem sequences' were recorded individually, and therefore, without information on the spatial relationship between them. The existence of 'aggregations' of short 'stem

sequences', in association with a minority of long 'stem sequences', was however, very apparent.

6.3.1.1 THE LENGTHS OF 'STEM SEQUENCES'

How frequently did the various lengths of 'stem sequences' occur?

	Internodes in 'stem sequence'													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Frequency	80	36	11	15	4	4	5	4	2	2	1	0	1	2

Table 6.38 The frequency of occurrence of different length 'stem sequences'

Table 6.38 shows that the vast majority of 'stem sequences' were very short, and that only a very small minority were long.

6.3.1.2 INTERNODE LENGTHS IN RELATION TO THEIR NUMERICAL POSITION WITHIN A 'STEM SEQUENCE'

Was there a relationship between the length of an internode and its numerical position within a 'stem sequence'?

Numerical position of internode within 'stem sequence'	Length of internode						
	3	5	7	9	11	13	15
14		2					
13		3					
12	1	1	1				
11		5					
10		7					
9		8					
8	3	9					
7		17					
6	4	16	1				
5	1	24					
4	3	34	3				
3	8	36	4	3			
2		70	14	3	1	1	
1	40	54	37	21	12	3	1

Table 6.39 The lengths of complete internodes by their numerical position within a 'stem sequence'

Table 6.39 shows that distally positioned internodes, within a 'stem sequence', were largely of five autozooids; and that both longer and shorter internodes, were essentially restricted to proximal positions.

6.3.1.3 INTERNODE LENGTHS AND THEIR SEQUENCE IN 22 LONG 'STEM SEQUENCES'

The lengths of internodes and their sequence within them were recorded for 22 long 'stem sequences' taken from five separate colonies. The material was separated into 'stem sequences', and numerical position does not equal internode generation.

Did these long 'stem sequences' exhibit any particular characteristics in terms of the lengths and/or sequences of their constituent internodes?

Numerical position of internode	Internode lengths and their sequence										
15										5	5
14										5	5
13				5						5	3
12		5		5						5	7
11		5	5	5			5		5	3	5
10	5	5	5	3	5	5	5	5	5	5	3
9	5	5	3	5	3	5	5	5	5	3	5
8	3	3	5	3	5	3	3	5	5	5	5
7	5	5	5	5	5	5	5	5	5	3	5
6	5	3	3	5	3	5	5	3	3	5	3
5	5	3	3	3	5	3	5	3	5	3	5
4	5	3	5	5	3	5	3	3	5	5	3
3	3	3	3	3	5	3	5	3	5	3	3
2	5	3	5	5	3	5	5	3	3	5	5
1	3	3	3	3	5	3	3	3	3	3	3
S/S number	1	2	3	4	5	6	7	8	9	10	11

Table 6.40 Internode lengths and their sequence within 22 long 'stem sequences' from five separate colonies. The numerical position is from the base, and is not internode generation.

(This Table continues, for 'stem sequences' originating in the subsequent 11 generations of internodes on the following page).

Numerical position of internode	Internode lengths and their sequence										
19										5	
18										5	
17	5					5				5	
16	5					5				5	
15	5				5	5				5	
14	5	5	5		5	3				5	5
13	3	5	5		5	5				3	5
12	5	5	5	5	5	5		5	5	5	3
11	3	5	5	5	3	5	5	3	5	5	5
10	5	3	3	5	5	5	5	5	3	3	5
9	5	5	5	3	5	5	5	3	5	5	3
8	5	3	5	5	3	3	5	5	3	5	5
7	3	5	5	5	5	5	3	3	3	3	3
6	5	3	5	3	5	3	5	3	3	5	5
5	3	5	3	5	3	5	5	5	3	3	3
4	5	3	5	5	5	3	5	3	3	5	5
3	5	5	3	5	3	5	5	3	3	3	3
2	5	5	5	3	5	3	5	3	3	5	5
1	3	3	3	3	3	5	3	3	3	3	3
S/S number	12	13	14	15	16	17	18	19	20	21	22

Table 6.40 (cont.) Internode lengths and their sequence within 22 long 'stem sequences' from five separate colonies. The numerical position is from the base, and is not internode generation.

Table 6.40 shows that long 'stem sequences' were essentially constituted of internodes of three and five autozooids; there was just one of seven. It thus confirms the results in respect of the four long 'stem sequences' of the detailed study. The frequent, but not constant, alternation of internodes of three and five noted there is also apparent here.

6.3.2 The distribution of lateral avicularia and ovicells in two long 'stem sequences' and all of the internodes which developed from them.

The detailed study investigated certain parameters in relation to the presence or absence of lateral avicularia and ovicells in a single colony. However, it ignored two aspects:-

- Firstly, no attempt was made to relate the actual presence or absence of either to the precise spatial arrangement of internodes within the colony.
- Secondly, the fact that lateral avicularia occurred in a variety of sizes had been ignored.

It was felt desirable to make good these deficiencies, and confirm or refute the findings of the detailed study. Two long 'stem sequences', together with all of the internodes which developed from them, were taken as representative samples from two separate colonies. In one, the material was only part of a colony, and I did not know the actual generations of the internodes; hence 'X', 'X'+1... In the second 'aggregation', the long 'stem sequence' originated in generation 'H'. In the results below, the two aggregations will be designated Aggregations 1 and 2, respectively.

(The overall spatial arrangement of internodes relative to one another, the numbers of their constituent autozooids, and the lateral avicularia and ovicells in relation to their autozooid of origin, for Aggregation 2, are shown in Figure 6.5).

- One square represents one autozooid.
- Incomplete internodes are indicated by a dot above the most distal autozooid.

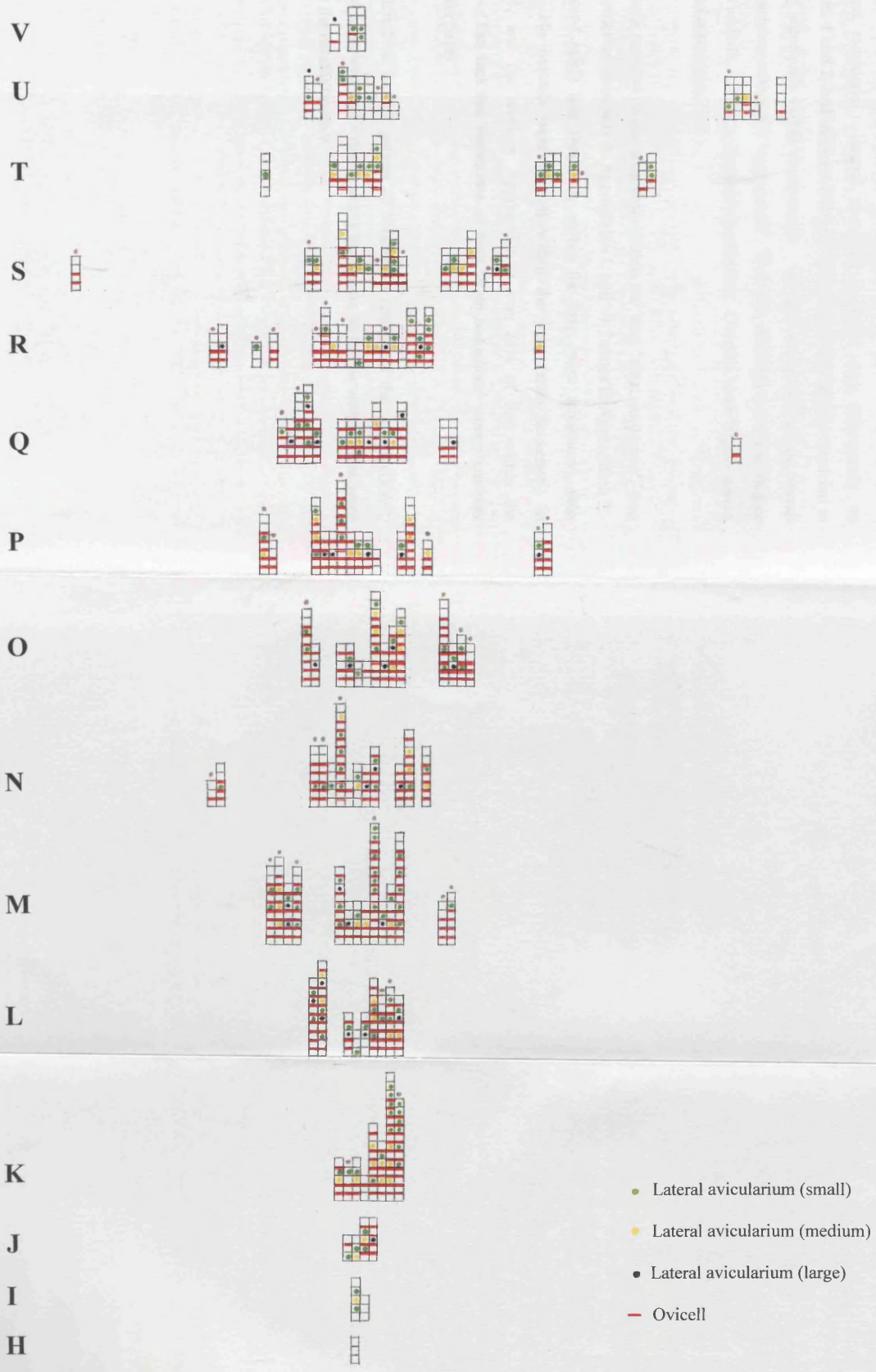


Figure 6.5 The pattern of internode presence and absence (and their length) in Aggregation '2', together with that of lateral avicularia and ovicells.

Figure 6.5 shows that lateral avicularia exhibited no overall pattern of occurrence within the 'aggregation'. They did exhibit a noticeably clumped distribution; internodes with lateral avicularia usually had more than one, and laterally, especially in the more distal generations, several internodes side by side would be with, or without, lateral avicularia. Lateral avicularia occurred very infrequently on autozooids Nos. 1 and 2. and medium and large forms were very largely restricted to odd-numbered autozooids within an internode. Ovicells also, exhibited no overall pattern of occurrence within the 'aggregation'. They did, although to a lesser degree than lateral avicularia, exhibit a clumped distribution. Ovicells were largely absent from sub-apical autozooids.

Lateral avicularia occurred more frequently within the long 'stem sequences', than outside them; twice as frequently in Aggregation 1, and ~1.5 times in Aggregation 2. Ovicells occurred much less frequently within the long 'stem sequences', than outside them. No ovicells were present within the long 'stem sequence', in Aggregation 1, and the level in Aggregation 2 was 20% of that within the 'aggregation'. (The fact that internodes of long 'stem sequences' were short was certainly a factor here).

Having considered the overall pattern of occurrence relative to the 'aggregations', how did lateral avicularia and ovicells occur in relation to the parameters that were investigated in the detailed study?

6.3.2.1 LATERAL AVICULARIA

Was lateral avicularia presence or absence related to internode generation?

Aggregation 1			
Internode Generation	Lateral avicularia		Percentage occurrence
	Present	Absent	
X +10	4	13	24
X + 9	9	40	18
X + 8	10	33	23
X + 7	16	44	27
X + 6	19	35	35
X + 5	15	43	26
X + 4	18	23	44
X + 3	11	16	41
X + 2	6	3	67
X + 1	3	1	75
X	2	0	100
Total	113	251	31

Aggregation 2			
Internode generation	Lateral avicularia		Percentage occurrence
	Present	Absent	
22	3	7	30
21	13	28	32
20	19	33	37
19	22	46	32
18	19	50	28
17	23	45	34
16	23	45	34
15	23	46	33
14	21	40	34
13	36	56	39
12	25	28	47
11	25	27	48
10	7	5	58
9	3	3	50
8	0	2	0
Total	262	461	36

Aggregation '1'-Chi-Square 24.783 P= 0.006 (5/23)
Aggregation '2'- Chi-Square 14.314 P= 0.427

Table 6.41 Lateral avicularia presence or absence by internode generation (apical autozooids and 'x' and 'i' cases excluded)

Table 6.41 shows that the two 'aggregations' were very different. In both however, the percentage occurrence of lateral avicularia fell at a certain stage, and did not regain its earlier level, although this was more marked in 'aggregation 1'.

Was lateral avicularia presence or absence related to whether an internode was a 'stem' or a 'branch'?

Internode type	Aggregation 1			Aggregation 2		
	Lateral avicularia		Percentage occurrence	Lateral avicularia		Percentage occurrence
	Present	Absent		Present	Absent	
'stem'	82	121	40	154	206	43
'branch'	31	130	19	108	255	30
Total	113	251	31	262	461	36

Aggregation '1'- Chi-Square, Yates' Correction for Continuity 17.769 P= < 0.001
Aggregation '2'-Chi-Square, Yates' Correction for Continuity 12.715 P= < 0.001

Table 6.42 Lateral avicularia presence or absence by 'stem' or 'branch' internodes (apical autozooids and 'x' and 'i' cases excluded)

Table 6.42 shows that lateral avicularia occurred substantially more frequently in 'stem' than 'branch' internodes, although not to the same extent in the two 'aggregations'.

Was lateral avicularia presence or absence related to whether an internode was complete or incomplete?

Internode type	Aggregation 1			Aggregation 2		
	Lateral avicularia		Percentage occurrence	Lateral avicularia		Percentage occurrence
	Present	Absent		Present	Absent	
Complete	102	179	36	202	304	40
Incomplete	11	72	13	60	157	28
Total	113	251	31	262	461	36

Aggregation '1'-Chi-Square, Yates' Correction for Continuity 14.839 P= < 0.001
Aggregation '2'-Chi-Square, Yates' Correction for Continuity 9.373 P= 0.002

Table 6.43 Lateral avicularia presence or absence by complete and incomplete internodes (apical autozooids and 'i' and 'x' cases excluded)

Table 6.43 shows that lateral avicularia occurred more frequently in complete than incomplete internodes, but to a very different extent in the two 'aggregations'..

Was lateral avicularia presence or absence related to autozooid position within an internode?

Autozooid position	Aggregation 1			Aggregation 2		
	Lateral avicularia		Percentage occurrence	Lateral avicularia		Percentage occurrence
	Present	Absent		Present	Absent	
Sub-apical	92	67	58	153	58	73
Proximal	18	180	9	91	392	19
Total	110	247	31	244	450	35

Aggregation '1'- Chi-Square, Yates' Correction for Continuity 96.117 P= < 0.001
Aggregation '2'- Chi-Square, Yates' Correction for Continuity 183.207 P= < 0.001

Table 6.44 Lateral avicularia presence or absence by autozooid position within an internode (apical autozooids and 'x' and 'i' cases excluded)

Table 6.44 shows that lateral avicularia occurred much more frequently on sub-apical than proximal autozooids, but to a very different extent in the two 'aggregations'.

Was lateral avicularia presence or absence related to autozooid number?

(Although lateral avicularia probably occur in a continuous range of sizes, I felt that some information could be obtained by dividing them by eye, into 'small', 'medium' and 'large', since a certain pattern of occurrence was apparent).

Aggregation 1						
Autozooid number	Lateral avicularia					Percentage occurrence
	Present				Absent	
	Small	Medium	Large	Total		
8	0	0	0	0	1	0
7	1	0	0	1	2	33
6	3	0	0	3	8	27
5	4	4	0	8	6	57
4	21	6	0	27	37	42
3	7	16	21	44	26	63
2	22	0	0	22	78	22
1	5	3	0	8	93	8
Total	63	29	21	113	251	31

Aggregation 2						
Autozooid number	Lateral avicularia					Percentage occurrence
	Present				Absent	
	Small	Medium	Large	Total		
14	2	0	0	2	0	100
13	2	0	0	2	0	100
12	2	0	0	2	1	50
11	4	1	0	5	0	100
10	2	1	0	3	4	43
9	6	1	1	8	0	100
8	6	0	0	6	15	29
7	9	11	3	23	1	96
6	13	0	1	14	27	32
5	25	12	5	42	5	88
4	39	0	0	39	84	30
3	35	42	26	103	29	78
2	9	0	0	9	147	6
1	3	1	0	4	148	3
Total	157	69	36	262	461	36

Aggregation '1'- Chi-Square 173.127 P= < 0.001 (16/50)
Aggregation '2'- Chi-Square 492.555 P= < 0.001 (32/57)

Table 6.45 Lateral avicularia presence or absence by autozooid number (apical autozooids and 'j' and 'x' cases excluded)

Table 6.45 shows that although lateral avicularia occurred at a very different level in the two 'aggregations', in both their occurrence was related to autozooid number. They occurred very infrequently on autozooids Nos. 1 and 2. They occurred more frequently on the odd-numbered autozooid of each staggered pair, except for the first where the opposite was the case. The larger forms occurred predominantly on autozooid number three (all occurred on this number autozooid in internodes of five autozooids, i.e. on the external of the sub-apical pair); and all but one occurred on odd-numbered autozooids. Table 6.44, above, showed that lateral avicularia occurred much more frequently on sub-apical autozooids. It was apparent, although not from these data (See Figure 6.5), that the lateral avicularium on the external of the sub-apical pair of autozooids was invariably the larger. (It is also clear from that figure that lateral avicularia did occur, if less frequently, on ovicellate autozooids; and that, the medium and large forms, were largely restricted to non-ovicellate autozooids).

Was lateral avicularia occurrence related to odd and even-numbered autozooids?

Autozooid number	Aggregation 1			Aggregation 2		
	Lateral avicularia		Percentage occurrence	Lateral avicularia		Percentage occurrence
	Present	Absent		Present	Absent	
Odd Nos.	61	126	32.6	187	183	50.5
Even Nos.	52	124	29.5	75	278	21.2
Total	113	250	31.1	262	461	36.2

Aggregation 1- Chi-Square, Yates' Correction for Continuity .269 P= 0.527
Aggregation 2- Chi-Square, Yates' Correction for Continuity 65.830 P= < 0.001

Table 6.46 Lateral avicularia occurrence by odd and even-numbered autozooids (apical autozooids and 'x' and 'i' cases excluded)

Table 6.46 shows that the two 'aggregations' were very different. Lateral avicularia occurred with equal frequency on odd and even-numbered autozooids in 'aggregation 1', but in 'aggregation 2' they occurred more than twice as frequently on odd-numbered autozooids.

Was there any relationship between the presence or absence of lateral avicularia and the presence or absence of ovicells?

Ovicell	Aggregation 1			Aggregation 2		
	Lateral avicularia		Percentage occurrence	Lateral avicularia		Percentage occurrence
	Present	Absent		Present	Absent	
Present	14	156	8	112	361	24
Absent	95	86	52	116	76	60
Total	109	242	31	228	437	34

Aggregation '1'- Chi-Square, Yates' Correction for Continuity 78.121 P= < 0.001
Aggregation '2'- Chi-Square, Yates' Correction for Continuity 80.186 P= < 0.001

Table 6.47 Lateral avicularia presence or absence related to the presence or absence of ovicells (apical autozooids and 'x' and 'i' cases excluded0

Table 6.47 shows that lateral avicularia occurred much more frequently on non-ovicellate autozooids, although to a different extent in the two 'aggregations'.

6.3.2.2 OVICELLS

Was ovicell occurrence related to internode generation?

Aggregation 1			
Internode Generation	Ovicell		Percentage Occurrence
	Present	Absent	
X + 10	4	17	19
X + 9	26	26	50
X + 8	24	29	45
X + 7	35	30	54
X + 6	30	35	46
X + 5	35	23	60
X + 4	16	19	46
X + 3	15	12	56
X + 2	0	8	0
X + 1	0	4	0
X	0	2	0
Total	185	205	47

Aggregation 2			
Internode generation	Ovicell		Percentage occurrence
	Present	Absent	
22	2	3	40
21	13	25	34
20	13	38	25
19	32	31	51
18	34	26	57
17	48	19	72
16	54	12	82
15	57	11	84
14	51	7	88
13	79	9	90
12	44	6	88
11	42	8	84
10	8	4	67
9	0	6	0
8	0	2	0
Total	477	207	70

Aggregation '1'- Chi-Square 25.403 P= 0.005 (6/28)
Aggregation '2'- Chi-Square 158.415 P= < 0.001 (7/24)

Table 6.48 Ovicell presence or absence by internode generation (apical autozooids and 'x' and 'i' cases excluded)

Table 6.48 shows that the level of ovicell occurrence was quite different in the two 'aggregations'. In both, however, they were initially absent and were then produced at a consistent level for a number of generations. In 'Aggregation 2' the level of occurrence declined in the distalmost generations.

Was ovicell occurrence related to the length of complete internodes?

Internode length, number of autozooids	Aggregation 1			Aggregation 2		
	Ovicell		Percentage occurrence	Ovicell		Percentage occurrence
	Present	Absent		Present	Absent	
15				12	2	86
13				11	0	100
11				16	1	94
9				66	10	87
7	34	20	63	63	22	74
5	70	116	38	130	137	49
3	2	62	3	5	20	20
Total	106	198	35	303	192	61

Aggregation '1'- Chi-Square 47.791 P= < 0.001
Aggregation '2'- Chi-Square 80.772 P= < 0.001 (1/7)

Table 6.49 Ovicell presence or absence by length of complete internodes (apical autozooids and 'x' and 'i' cases excluded)

Table 6.49 shows that the longer the internode the higher the level of ovicell occurrence. The fact that ovicells occurred much more frequently on proximally sited autozooids, 11 times more frequently in 'aggregation 1' and >3 times in 'aggregation 2' (see Table 6.52) was clearly a factor.

Was ovicell occurrence related to whether an internode was a 'stem' or a 'branch'?

Internode type	Aggregation 1			Aggregation 2		
	Ovicell		Percentage occurrence	Ovicell		Percentage occurrence
	Present	Absent		Present	Absent	
'stem'	88	134	40	207	127	62
'branch'	97	71	58	270	80	77
Total	185	205	47	477	207	70

Aggregation '1'- Chi-Square, Yates' Correction for Continuity 11.847 P= 0.001
Aggregation '2'- Chi-Square, Yates' Correction for Continuity 17.916 P= < 0.001

Table 6.50 Ovicell presence or absence by 'stem' and 'branch' internodes (apical autozooids and 'x' and 'i' cases excluded)

Table 6.50 shows that ovicells occurred more frequently in 'branches' than 'stems', but to a different extent in the two 'aggregations'.

Was ovicell occurrence related to whether an internode was complete or incomplete?

Internode type	Aggregation 1			Aggregation 2		
	Ovicell		Percentage occurrence	Ovicell		Percentage occurrence
	Present	Absent		Present	Absent	
Complete	106	198	35	303	192	61
Incomplete	79	7	92	174	15	92
Total	185	205	47	477	207	70

Aggregation '1'- Chi-Square, Yates' Correction for Continuity 85.054 P= < 0.001
Aggregation '2'- Chi-Square, Yates' Correction for Continuity 60.232 P= < 0.001

Table 6.51 Ovicell presence or absence by complete and incomplete internodes (apical autozooids and 'x' and 'i' cases excluded)

Table 6.51 shows that although the situation was very different in the two 'aggregations', in both, they occurred on >90% of autozooids in incomplete internodes. The level of occurrence in complete internodes was almost twice as high in 'aggregation 2'. (Although not apparent from the table, incomplete internodes were often completely ovicellate).

Was ovicell occurrence related to autozooid position within an internode?

Autozooid position	Aggregation 1			Aggregation 2		
	Ovicell		Percentage occurrence	Ovicell		Percentage occurrence
	Present	Absent		Present	Absent	
Sub-apical	13	162	7	49	144	25
Proximal	170	43	80	428	56	88
Total	183	205	47	477	200	70

Aggregation '1'- Chi-Square, Yates' Correction for Continuity 199.094 P= < 0.001
Aggregation '2'- Chi-square, Yates' Correction for Continuity 260.426 P= < 0.001

Table 6.52 Ovicell presence or absence by autozooid position within an internode (apical autozooids and 'x' and 'i' cases excluded)

Table 6.52 shows that ovicells occurred much more frequently on proximal than sub-apical autozooids, but to a very different extent in the two 'aggregations'.

Was ovicell occurrence related to autozooid number within an internode?

Aggregation 1			
Autozooid number	Ovicell		Percentage occurrence
	Present	Absent	
7	1	1	50
6	3	9	25
5	5	11	31
4	32	38	46
3	28	45	38
2	63	45	58
1	53	56	49
Total	185	205	47

Aggregation 2			
Autozooid number	Ovicell		Percentage occurrence
	Present	Absent	
14	0	1	0
13	0	1	0
12	2	0	100
11	3	0	100
10	6	0	100
9	6	1	86
8	15	3	83
7	15	6	71
6	29	8	78
5	30	20	60
4	70	41	63
3	56	62	47
2	126	25	83
1	119	39	75
Total	477	207	70

Aggregation '1'- Chi-Square 11.811 P= 0.066
Aggregation '2'- Chi-Square 61.258 P= < 0.001 (12/43)

Table 6.53 Ovicell presence or absence by autozooid number (apical autozooids and 'x' and 'i' cases excluded)

Table 6.53 shows no evidence that ovicell occurrence was related to autozooid number in any consistent way.

Was ovicell occurrence related to odd and even-numbered autozooids?

Autozooid number	Aggregation 1			Aggregation 2		
	Ovicell		Percentage occurrence	Ovicell		Percentage occurrence
	Present	Absent		Present	Absent	
Odd Nos.	87	113	43.5	229	123	65.1
Even Nos.	98	92	51.6	248	78	76.1
Total	185	205	47.4	477	201	70.4

Aggregation 1- Chi-Square, Yates' Correction for Continuity 2.237 P= 0.135
Aggregation 2 - Chi-Square, Yates' Correction for Continuity 9.328 P= 0.002

Table 6.54 Ovicell occurrence by odd and even-numbered autozooids (apical autozooids and 'x' and 'i' cases excluded)

Table 6.54 shows that although the level of ovicell occurrence was very different in the two 'aggregations', in both ovicells occurred somewhat more frequently on even-numbered autozooids.

Was there any relationship between the presence or absence of ovicells, and the presence or absence of lateral avicularia?

Lateral avicularia	Aggregation 1			Aggregation 2		
	Ovicell		Percentage occurrence	Ovicell		Percentage occurrence
	Present	Absent		Present	Absent	
Present	14	95	13	112	116	49
Absent	156	86	64	361	76	83
Total	170	181	48	473	192	71

Aggregation '1'- Chi-Square, Yates' Correction for Continuity 78.121 P= < 0.001
Aggregation '2'- Chi-Square, Yates' Correction for Continuity 80.186 P= < 0.001

Table 6.55 Ovicell presence or absence related to the presence or absence of lateral avicularia (apical autozooids and 'x' and 'i' cases excluded)

Table 6.55 shows that ovicells occurred much more frequently on autozooids without lateral avicularia, but again the difference between the two 'aggregations' was considerable.

For 'aggregation 2' I related the presence or absence of lateral avicularia and ovicells, to their level of occurrence, and internode generation. The results are shown in Table 6.56, below:-

Internode generation	Lateral avicularia only	Ovicell only	Both present	Both absent
V	3	2	0	2
U	8	12	0	14
T	18	13	0	19
S	16	29	3	16
R	14	32	2	12
Q	14	40	7	4
P	12	43	10	1
O	8	45	13	1
N	4	35	15	2
M	5	53	27	1
L	4	24	19	1
K	7	27	15	0
J	4	5	3	0
I	3	0	0	3
H	0	0	0	2
Total	120	360	114	78

Table 6.56 Lateral avicularia and ovicell presence or absence by internode generation, in an 'aggregation' of internodes developed from a long 'stem sequence' (apical autozooids and 'x' and 'i' cases excluded)

From Table 6.56 it appears that lateral avicularia and ovicells may occur on the same autozooid, only when the numbers of both produced were at such levels that it was impossible for them to be on separate autozooids.

6.3.3 The presence/absence of lateral avicularia and ovicells in long 'stem sequences'

In the detailed study described in Section 6.2, observations were made in respect of the presence or absence of lateral avicularia and ovicells within the four longest 'stem sequences' of that colony. They were compared with the situation obtaining within the colony overall. (See Table 6.23 re lateral avicularia, and Table 6.35 re ovicells). The numbers involved in both cases were very small, and it was necessary to investigate these aspects more fully (see Section 4.5.5.2.2).

14 long 'stem sequences', each of 12 or more internodes, were extracted from a small number of colonies (each of which was densely ovicellate) and the presence or absence, and distribution, in certain respects, of lateral avicularia and ovicells recorded.

6.3.3.1 LATERAL AVICULARIA

How did the frequency of lateral avicularia occurrence, within these long 'stem sequences', compare with that of the complete colony considered earlier?

Table 6.15 showed that, within the complete colony, lateral avicularia occurred on 27.5% of those autozooids which could give rise to them. Within the four longest 'stem sequences', they occurred on 67% of such autozooids. In the 14 long 'stem sequences' investigated here, of the 611 autozooids which could produce lateral avicularia, 334 (55%) did so.

Was the level of lateral avicularia occurrence similar in each of the long 'stem sequences', and in their colonies of origin?

Colony number	'Stem sequence' number	Lateral avicularia		Percentage occurrence
		Present	Absent	
1	1	27	3	90
1	2	24	15	62
2	3	28	20	58
2	4	27	22	55
2	5	25	30	45
2	6	20	22	48
2	7	22	25	47
2	8	11	27	29
3	9	19	31	38
3	10	30	24	56
4	11	24	4	86
4	12	20	8	71
5	13	38	23	62
5	14	19	23	45
Total		334	277	55

Table 6.57 Lateral avicularia presence or absence in 14 long 'stem sequences' from five separate colonies (apical autozooids and 'x' and 'i' cases excluded)

Table 6.57 shows considerable variation in the level of lateral avicularia occurrence, between different 'stem sequences', from 29% to 90%; and to a lesser extent between different colonies.

Was presence or absence of lateral avicularia related to the position within the internode of the autozooid which gave rise to it?

Autozooid position	Lateral avicularia		Percentage occurrence
	Present	Absent	
Sub-apical	282	89	76
Proximal	52	188	22

Chi-Square, Yates' Correction for Continuity 170.854 P= < 0.001

Table 6.58 Lateral avicularia presence or absence by autozooid position within an internode (apical autozooids and 'x' and 'i' cases excluded)

Table 6.58 shows that lateral avicularia occurred 3.5 times more frequently on sub-apical than proximal autozooids.

Was lateral avicularia occurrence related to autozooid number?

Autozooid Number	Lateral avicularia		
	Present	Absent	Percentage occurrence
6	0	1	0
5	1	0	100
4	90	20	82
3	112	4	97
2	57	135	30
1	74	117	39

Table 6.59 Lateral avicularia presence or absence by autozooid number (apical autozooids and 'x' and 'i' cases excluded)

Table 6.59 shows that lateral avicularia occurred much more frequently on autozooids three and four than on autozooids one and two, and they occurred somewhat more frequently on the odd-numbered autozooid of each pair. (Not only did lateral avicularia occur more frequently on autozooid number three than number four, but the largest forms, invariably occurred only on this autozooid).

6.3.3.2 OVICELLS

How did the frequency of ovicell occurrence, within these long 'stem sequences', compare with that of the complete colony considered earlier?

Table 6.35 showed, that within that colony, ovicells occurred on 67% of those autozooids which could give rise to them, but in the four longest 'stem sequences' 130 such autozooids produced just one ovicell. In the 14 long 'stem sequences' considered here, of the 637 autozooids which could produce ovicells, 80 (13%), did so.

Was the rate of ovicell occurrence similar in each of the long 'stem sequences', and in their colonies of origin?

Colony number	'Stem sequence' number	Ovicell		Percentage occurrence
		Present	Absent	
1	1	0	34	0
1	2	0	42	0
2	3	2	45	4
2	4	6	44	12
2	5	11	47	23
2	6	10	32	24
2	7	12	36	25
2	8	13	25	34
3	9	11	39	22
3	10	7	51	12
4	11	0	32	0
4	12	0	32	0
5	13	3	59	5
5	14	5	39	11
Total		80	557	13

Table 6.60 Ovicell presence or absence in 14 long 'stem sequences' from five separate colonies (apical autozooids and 'x' and 'i' cases excluded)

Table 6.60 shows considerable variation in ovicell occurrence between 'stem sequences', and less between colonies. Four 'stem sequences', from two colonies, had none at all; five, from two colonies, had a level between 22% and 34%.

Was ovicell occurrence related to the autozooid position within an internode?

Autozooid position	Ovicells		Percentage occurrence
	Present	Absent	
Sub-apical	13	384	3
Proximal	67	173	28

Chi-Square, Yates' Correction for Continuity 80.481 P= < 0.001

Table 6.61 Ovicell presence or absence by autozooid position within an internode (apical autozooids and 'x' and 'i' cases excluded)

Table 6.61 shows that ovicells occurred nine times more frequently on proximally than sub-apical autozooids.

6.4 COLLATION AND SUMMARY OF RESULTS

6.4.1 Introduction

To avoid unnecessary repetition, where the situation here is identical to that in respect of *S. reptans,* detailed in Chapter 5, I shall refer to the results of that chapter. The full discussion of the results of both chapters constitutes Chapter 7. In this collation I shall bring together the results from the detailed and supplementary studies, and make clear the extent of any variation.

As in Chapter 5, this collation is in two sections. The first is concerned with autozooids, internodes, 'stem sequences', and colony structure and form. The second relates to the occurrence of polymorphs and ovicells.

The results are largely derived from the colony of the detailed study. Data from supplementary studies will be identified as such.

6.4.2 Autozooids in internodes; bifurcations; internodes within 'stem sequences', their arrangement within the colony, and its structure and form

6.4.2.1 AUTOZOOIDS WITHIN INTERNODES, AND BIFURCATIONS

As discussed in respect of *S. reptans,* in Section 5.5.2.1, internodes were distinguished in three respects: their length; whether they were complete or incomplete; and whether they were a 'stem' or a 'branch'.

The arrangement of autozooids within internodes was exactly the same as in *S. reptans* and all complete internodes were of an odd number of autozooids. Regarding the lengths of complete internodes, 80% were of three or five, with the latter occurring 25% more frequently than the former. Internodes of seven, nine and eleven also occurred, their numbers decreasing with increasing length.

The relationship between internode length and internode generation was shown in Table 6.2. The shortest internodes, of three autozooids, dominated the more proximal generations; in this colony no longer internodes were found until the sixth generation. Subsequently, internodes of three autozooids continued to occur, but they became proportionally less numerous with each subsequent generation. Internodes of five and seven autozooids became increasingly more important in the more distal generations of internodes, and longer internodes occurred sparsely throughout the central and more distal regions of the colony. The average length of internodes, beyond the first five generations, essentially increased generation on generation.

The arrangement of internodes at bifurcations, and the consistent pattern of branching, were also the same as for *S. reptans*, as described in Section 5.5.2.1.

Regarding complete and incomplete internodes, the situation was virtually identical to that for *S. reptans* with some 40% incomplete.

The total numbers, and the numbers of the various lengths of 'stem' and 'branch' internodes, were not as might have been expected. A very different pattern from that

observed in *S. reptans* was apparent. For complete internodes, there were 50% more 'stems' than 'branches', but neither of the two internode lengths which occurred in quantity, approximated to this 60/40 ratio. The percentage of 'stems', for internodes of three and five, were ~40% and 85%, respectively. For internodes with more than five autozooids, the longer the internode, the more likely it was to be a 'branch'. For incomplete internodes, there were 50% more 'branches' than 'stems'. In terms of occurrence and length, 'stems' and 'branches', did differ.

6.4.2.2 INTERNODES WITHIN 'STEM SEQUENCES'

The investigation into the 'stem sequences' of *S. reptans* revealed three features:-

- A small number of very long, and a very large number of short, or very short, 'stem sequences'.
- That the internode composition of the very long 'stem sequences', was very different from those of the complete colony.
- That for all 'stem sequences', internodes of other than five autozooids, were very largely confined to the most proximal positions within a 'stem sequence'.

Although the colony investigated in detail was not as large as that of *S. reptans* it did exhibit a small number of long, and a much larger number of short, 'stem sequences'. Over 93% of 'stem sequences' were of five or fewer internodes, and less than 2% were of 11 or more. A supplementary study on much of a larger colony produced almost identical percentage occurrences.

The internode constitution of long 'stem sequences' was different from that of *S. reptans* in that it consisted of internodes of three (~ 60%) and five autozooids (~40%). Within the colony, the percentages were 36% and 44% respectively, and 20% of internodes, were of seven, nine or eleven autozooids. A supplementary study of a number of somewhat longer long 'stem sequences' than those in the colony of the detailed study, produced similar results, but with internodes of five outnumbering those of three, two to one. Internodes of three were more numerous in proximal, and those of five in more distal generations

As for *S. reptans,* there was a possibility that the particular internode composition of the long 'stem sequences' was a secondary feature, resulting from known differences between them and the colony. Firstly, the proportions of the different internode lengths in the various generations of internodes were very different in the two. Secondly, the long 'stem sequences' were, by definition, composed almost entirely of 'stem' internodes, whilst within the colony, there were only slightly more 'stems' than 'branches'. In Section 6.2.2.1.4.3, it was demonstrated that neither of these characteristics could be invoked to explain completely the difference between the internode composition of the long 'stem sequences' and the colony. The figures were not as clear-cut as those for the colony of *S. reptans,* which was much better developed. In the material used in the supplementary studies, and a number of unrecorded observations, distally discrete 'aggregations' of internodes in short 'stem sequences' associated with, and centred on, long 'stem sequences', were characteristic.

For all internodes, their length, and their numerical position within the 'stem sequence' in which they occurred were recorded (Table 6.8). This showed, that internodes of other than five autozooids, especially those which were longer, were largely confined to the first or second positions, within a 'stem sequence'. A supplementary study of a substantial part of a large colony (Table 6.39) confirmed that 85% of internodes beyond the second position within a 'stem sequence' were of five autozooids, although they constituted only 53% of all internodes.

6.4.2.3 THE ARRANGEMENT OF INTERNODES AND 'STEM SEQUENCES' WITHIN A COLONY, AND THE STRUCTURE AND FORM WHICH RESULTS

As for *S. reptans,* the long 'stem sequences' again generally originated in the more proximal generations of internodes. One long 'stem sequence' in one of the supplementary studies, however, had its origin in generation eight, and therefore there was no direct link between the generation of origin of a 'stem sequence' and its potential length.

Although the colony was probably still growing when collected, it was clear from Table 6.1 that the theoretically possible doubling of the number of internodes in each

successive generation ceased to occur in the fifth generation, and that internode numbers thereafter increased only slowly and then declined. Complete internodes showed a pattern of increase and decrease in number over the generations. Incomplete internodes were completely absent from the first four generations, and their number, as a proportion of the number within a generation, essentially increased generation on generation.

As shown in section 6.2.2.1.4.4, it was apparent that the four longest 'stem sequences' of the detailed study were far from evenly spaced laterally within the colony. Their lateral arrangement suggested a colony much influenced by available space or damage. Their distribution was illustrated diagrammatically in Figure 6.3, which also showed the position of one 'stem sequence', which was not particularly long, but which had the internode composition of such a long 'stem sequence' (possibly broken).

As for *S. reptans*, all possible internodes developed in the proximal region of the colony, but as growth proceeded there was a large scale 'thinning-out', which increased in magnitude in the more distal regions.

As discussed in Section 6.2.2.1.4.4, the clustering of internodes beyond the proximal generations of internodes, around internodes within the long 'stem sequences', was very apparent. It resulted in a limited number of vertically extensive 'aggregations' of internodes, which distally, were laterally discrete.

Diagrammatic representation of a single long 'stem sequence', and the internodes which developed from it (over a number of generations) showed that these 'aggregations' were laterally very limited. Internodes were very largely concentrated in a narrow, probably potentially lanceolate, vertical band, centred on a long 'stem sequence'.

Such 'aggregations' of internodes, developing from long 'stem sequences', are not, of course, two-dimensional. The angulation of internodes in relation to one another at bifurcations, and to a lesser degree the lengthwise concavity of the longer internodes, results in incurving, both distally and laterally. These characteristics are

more pronounced in *T. inopinata* than *S. reptans,* and with 'aggregations' often forming semi-clenched fist-like structures, the colony is generally altogether denser. The overall three-dimensional structure of the colony, which results, is essentially an incomplete circle of these structures which develop from a vertically limited, laterally continuous, proximal region.

6.4.3 The spatial arrangement of polymorphs and ovicells

6.4.3.1 INTRODUCTION

The situation was less complex than it was for *S. reptans,* partly because material from only one population was considered; although there was some evidence of inter-colony variation, particularly in respect of ovicells. There were also fewer polymorphs to consider, and also therefore, fewer possible associations between them. It was known that the most proximal external spine was sometimes bifid, and that scuta varied greatly in morphology. The condition of the material did not, unfortunately, allow an investigation into any possible pattern of occurrence of these characteristics individually, together, or perhaps with a third element, ovicells.

The introductory remarks made in Section 5.5.3.1, to the collation and summary of results, regarding polymorphic zooids in *S. reptans,* are equally applicable here.

6.4.3.2 THE SPATIAL ARRANGEMENT OF POLYMORPHS AND OVICELLS WITHIN A COLONY

The spatial occurrence of polymorphs can be distinguished on the basis of whether they occurred constantly, predictably, or unpredictably.

Spines and scuta occur, as was already known, constantly on all autozooids, except the ancestrula, in a consistent fashion, in relation to the position of their autozooid of origin, within an internode. Variations in morphology were referred to above.

6.4.3.2.1 Lateral avicularia

Lateral avicularia occurred unpredictably, but their spatial disposition exhibited a number of characteristics:-

- Avicularia occurrence was related to internode generation. In the colony of the detailed study, they were numerous in the first six generations and then exhibited a sudden decline, firstly to 50% of their previous level and then lower still. One of the 'aggregations' of the supplementary study showed a similar pattern, whilst the trend was less obvious in the second.
- Avicularia occurred virtually twice as frequently in 'stem' than 'branch' internodes, in the detailed study, and one of the 'aggregations' of the supplementary study. In the second the difference was somewhat smaller.
- Avicularia occurred almost four times more frequently in complete, than incomplete internodes in the colony of the detailed study. In the 'aggregations' of the supplementary study the asymmetry was not as great, but still substantial.
- Avicularia occurrence was not related to internode length.
- Avicularia occurred ~6 x more frequently on sub-apical, as opposed to proximal autozooids in the colony of the detailed study, and in the first 'aggregation'. In the second 'aggregation' they occurred ~4 x more frequently. Large forms occurred almost completely on the odd-numbered autozooid of the sub-apical pair.
- In the colony of the detailed, and the 'aggregations' of the supplementary study, avicularia occurrence showed a definite, if complex, relationship to autozooid number. They occurred infrequently on autozooids Nos. 1 and 2 (and twice as frequently on the latter) but thereafter were more numerous on the odd-numbered autozooid of each pair. This trend was increasingly apparent as autozooid number increased. Overall avicularia occurred with equal frequency on odd and even-numbered autozooids in the colony of the detailed study and in 'aggregation 1'. In 'aggregation 2' they occurred 2.5 x more frequently on odd-numbered autozooids.
- Avicularia occurrence was related to length of 'stem sequence'; the longer the 'stem sequence' the higher the level of avicularia occurrence.

Lateral avicularia were thus positively correlated with:-

- Internodes within proximal generations.
- Complete internodes.
- 'Stem' internodes.
- Sub-apical autozooids.
- Odd-numbered autozooids, excluding No. 1.
- Longer length 'stem sequences'

When lateral avicularia presence or absence was related to both autozooid position within an internode and internode generation (see Table 6.22), it appeared, although numbers were very small, that the latter was only a factor in respect of proximally positioned autozooids. On sub-apical autozooids lateral avicularia occurred at a high percentage of available sites. They occurred six times more frequently on sub-apical as opposed to proximal autozooids. Further the very largest forms occurred only on the externally positioned of this pair. The situation in the supplementary studies was again slightly less clear-cut. Lateral avicularia production was, therefore, in two respects, number and size, heavily concentrated on the sub-apical autozooids, especially the external.

6.4.3.2.2 Rhizoids

Rhizoids, as has long been known, were strongly concentrated vertically in proximal generations. They were also concentrated laterally within long 'stem sequences', and internodes adjacent to them.

6.4.3.2.3 Ovicells

Before considering the results in respect of the various parameters investigated in the detailed and supplementary studies, it is worth recalling one aspect that was investigated in Chapter 3. Non-ovicellate and ovicellate autozooids generally look 'different', and their basic dimensions were recorded. The results (see Table 3.1) show that, on average, non-ovicellate were ~20% longer than ovicellate autozooids. The results also show that the actual difference in autozooid length was reflected in an identical difference in the length of the opesia.

The detailed and supplementary studies showed that:-

- Ovicell occurrence level varied considerably between colonies, but they were absent from the first few generations of internodes, occurred consistently thereafter, and declined perhaps, only in the distalmost generations.
- Ovicells were virtually absent from the four longest 'stem sequences' of the colony of the detailed study, although it was abundantly ovicellate. Within fertile 'aggregations' of the supplementary study, ovicells were absent, or sparse, in long 'stem sequences'
- The longer the length of internode, the higher the level of ovicell occurrence.
- Ovicells occurred more than twice as frequently in incomplete internodes in the colony of the detailed study, and in one of the 'aggregations' in the supplementary study; and 1.5 x higher in the other.
- Ovicells occurred more frequently, in 'branch' than 'stem' internodes; by ~50% in the colony of the detailed study and in 'aggregation 1'; and by 25% in 'aggregation 2' of the supplementary study.
- In the detailed study ovicell presence or absence was strongly correlated with autozooid position within an internode; positively so with 'proximal', 88% of which were ovicellate, and negatively with sub-apical, 93% of which were not. The situation was very similar in 'aggregation 1', but the difference was ~3.5 x in 'aggregation 2'.
- In the detailed study, ovicells occurred equally on odd and even-numbered autozooids. In the two 'aggregations' of the supplementary study they occurred somewhat more frequently on even-numbered autozooids. .
- Ovicell occurrence was inversely related to the length of 'stem sequences'; occurring abundantly in short 'stem sequences' and being rare in, or absent from, long 'stem sequences'.

It is clear from the above, that in respect of the majority of the parameters considered, both lateral avicularia and ovicells exhibited marked, or very marked asymmetries of occurrence. These, whilst often varying in extent in the replicate studies, were always in the same direction. It is also very apparent, that in almost every respect, the 'preferred' site of one, was the 'less favoured' of the other. The relationship between the two will be considered below.

6.4.3.3 POSITIVE AND NEGATIVE CORRELATIONS BETWEEN VARIOUS POLYMORPHS, AND BETWEEN POLYMORPHS AND OVICELLS

There was only one such correlation here, between lateral avicularia and ovicells. This was an observed correlation, which may not, of course, be due solely to a positive or negative association between the two.

6.4.3.3.1 Lateral avicularia and ovicells

Lateral avicularia were negatively correlated with ovicells; they occurred almost six times more frequently on non-ovicellate autozooids, in the colony of the detailed study and in 'aggregation 1'. In 'aggregation 2' they occurred 2.5 x more frequently.

Ovicells occurred more than 3.5 x as frequently on autozooids that did not have lateral avicularia in the detailed study. In the 'aggregations' of the supplementary study they occurred 5 x more frequently in the first, but only by 60% in the second.

It is clear from Tables 6.15 and 6.27 that lateral avicularia occurrence decreased dramatically in the internode generation in which substantial ovicell production began. Tables 6.57 and 6.60 show that for the 14 long 'stem sequences', lateral avicularia occurrence was negatively correlated with the occurrence of ovicells.

The results of an investigation into the occurrence of lateral avicularia and ovicells in 'aggregation 2' of the supplementary studies were shown in Table 6.56. This suggests that the two may have occurred on the same autozooid, largely only when there were insufficient autozooids, for the numbers produced, to be produced by separate autozooids.

There was much evidence, in virtually all of the parameters investigated, that in *T. inopinata* lateral avicularia and ovicell occurrence were strongly negatively correlated. The results were slightly different for the three colonies.

- When lateral avicularia and ovicell occurrence was related to 'stem' and 'branch' internodes, lateral avicularia occurred twice as frequently in 'stems', whilst ovicells occurred ~50% more frequently in 'branches'.
- In relation to complete and incomplete internodes, lateral avicularia occurred ~four times more frequently in complete, whilst ovicells occurred twice as frequently in incomplete, internodes.
- When autozooid position was considered, lateral avicularia occurred four to six times more frequently on sub-apical autozooids whilst ovicells occurred >10 times more frequently on proximal autozooids, in the majority of the material studied.

Strictly speaking, these results relate the production of lateral avicularia and embryos, not ovicells. However the opening to an ovicell produced by an autozooid for an embryo produced by the autozooid proximal to it, is adjacent to any lateral avicularia produced by that autozooid.

6.4.3.3.2 Single/bifid spines and limited/extensive scuta

As discussed above, there may well be a correlation between simple and bifid spines and the extensiveness of scuta, and perhaps with the presence or absence of ovicells. The condition of the material did not, unfortunately, allow a quantitative investigation to be made.

6.4.4 Summary

There was no indication of any variation between colonies in respect of the arrangement of autozooids within internodes, internodes within 'stem sequences', or 'stem sequences' within a colony.

All internodes were of an odd number of autozooids, resulting in asymmetrical bifurcations, and a consistent branching pattern. Spatial constraints mean that not all internodes bifurcate. Differentiating between 'stems' and 'branches' at bifurcations, led to the concept of 'stem sequences'. A colony consisted of a small number of long, and a large number of much shorter, 'stem sequences'. All 'stem sequences',

develop in a shallowly sinusoidal manner, with an essentially constant direction of growth, and with 'branches' developing alternately to left and right. The considerable lateral variation in the extent of vertical growth, even in the absence of external constraints, was clearly related to the existence of a limited number of long 'stem sequences'; with growth, beyond the more proximal region, only developing, directly or indirectly, from them. Long 'stem sequences' differed from the shorter 'stem sequences' of the mass of the colony, in that their constituent internodes were generally shorter, with the longest lengths being completely absent. The spatial arrangement of the various length internodes exhibited an indefinite pattern; early generation internodes were exclusively of three autozooids, but internodes of five and longer increasingly superseded them in later generations. The fact that longer length internodes were essentially confined to the more proximally positioned internodes within a 'stem sequence' was the dominant element of the spatial arrangement of different length internodes. Long 'stem sequences' generally well-spaced, formed central ribs of lanceolate, 'aggregations' of internodes (within short 'stem sequences') which in the central and distal regions of the colony, were quite discrete. Each 'aggregation' was noticeably incurved, distally and laterally, often resembling a partially clenched fist. The resultant colony form, an incomplete circle of such structures, developing from a vertically limited, laterally continuous, proximal region, was generally dense.

Polymorphs can be said to occur constantly, predictably, or unpredictably. Mural spines and scuta occurred constantly on all autozooids, except the ancestrula; but both exhibited unpredictable morphological variation. The proximal external spine was sometimes bifid, and scuta occurred in a number of different morphologies. Lateral avicularia occurred unpredictably, their distribution being very complex. They were positively correlated with early generations of internodes, and with odd, high-numbered and sub-apical autozooids, within an internode. The largest forms were only present on the external sub-apical autozooid of an internode. Lateral avicularia also occurred much more frequently in complete, than incomplete internodes and, to a lesser extent, in 'stems' than 'branches'. They occurred more frequently in longer length 'stem sequences'. Lateral avicularia were strongly negatively correlated with the presence of ovicells. Rhizoids were concentrated vertically, in proximal generations, and laterally, within or close to, long 'stem

sequences'. Ovicellate autozooids were, on average, ~20% shorter than non-ovicellate. The level of ovicell occurrence varied between colonies, but was generally high. Ovicells were infrequent or absent, in early generations of internodes, within long 'stem sequences', and on sub-apical autozooids. They occurred more frequently in incomplete than complete internodes, in 'branches' than 'stems', and in short 'stem sequences'. They occurred much more frequently on autozooids which were without lateral avicularia.

CHAPTER 7 - CONCLUDING DISCUSSION

7.1 INTRODUCTION

The basic premise for the studies of *Scrupocellaria reptans* and *Tricellaria inopinata* was that the arrangement of zooids, autozooids and polymorphic heterozooids, within a colony, could be described in greater definition than was generally the case; and that this could reveal previously undescribed patterns, trends or correlations. The spatial arrangement of autozooids could reveal the gross structure of a colony.

The study investigated a number of parameters and possible relationships, many of which, so far as I am aware, had not previously been looked at in a quantitative way. There is therefore, for these, little pertinent information in the literature. In respect of *S. reptans*, however, Lutaud (1953) described its consistent branching pattern. There are few synoptic reviews of polymorphism within the Bryozoa; Silén's (1977) review being the most notable, and unfortunately the most recent, exception. There is an enormous amount of generally qualitative information on polymorphic heterozooids scattered throughout the taxonomic literature. Some of these published observations, particularly those concerning unilaminar, biserial, arborescent Bryozoa, together with those of Harmer (1923) and Hastings (1943) do resonate with results of this study and will be incorporated where appropriate. I also have limited information on a third such species, *Scrupocellaria scruposa.*

Theoretically, especially for non-encrusting species, autozooid modules could be spatially arranged in an almost infinite variety of ways. In fact, if upright forms are viewed in terms of their large-scale structural organization, a surprisingly small number of forms are to be found in the fossil record. The same forms, by and large, exist today (McKinney and Jackson, 1989). This, in itself, suggests that only certain spatial arrangements of modules have proved to be evolutionally successful. The various colony forms which have been described have been distinguished on the basis of large-scale characteristics, i.e. unilaminar and bilaminar, bifurcating and anastomosing, flexible and rigid. It is surely to be expected, that other characteristics of colony structure exist, as less obvious manifestations of the evolutionary process.

Whilst the study of a single species could reveal much about that species, it was also desirable to look at a second species to establish whether or not any newly revealed characteristics occurred more widely, and were therefore of greater significance. The discovery of *T. inopinata,* a species new to Britain, suggested itself as an ideal second species to investigate. It is very similar to *S. reptans,* in that both are unilaminar, biserial and arborescent forms. They had therefore, a number of characteristics in common which could be compared.

7.2 DISCUSSION

7.2.1 Introduction

This discussion brings together the results of the studies of *Scrupocellaria reptans* and *Tricellaria inopinata* (Chapters 5 and 6) and discusses the similarities and differences of their characteristics. A variety of parameters were investigated and numerous asymmetries of occurrence were apparent in respect of autozooids, internodes, 'stem sequences', polymorphic heterozooids and ovicells. It is probable that some of these asymmetries are secondary and it is important to distinguish these from primary characteristics.

I shall speculate where possible and appropriate on why certain characteristics might occur, and on their possible biological significance. Results of the studies which appear to lack obvious explanation will be identified. Finally, where an investigation has, in my view, suggested likely further lines of enquiry, these will be set out. The discussion will be centred on *S. reptans* and *T. inopinata* but broadened where this seems worthwhile.

The arrangement of autozooids within internodes and internodes within 'stem sequences' is fundamental in determining the structure of the colony. The spatial distribution of heterozooids, however numerous and varied they may be, is secondary. This discussion will deal firstly with autozooids, internodes, bifurcations, 'stems' and 'branches', 'stem sequences', and the structure and form of a colony. Secondly it will be concerned with the numbers and spatial arrangement of

heterozooids and the pattern of female reproductive zooids, as evidenced by the presence or absence of ovicells.

To minimize repetition, and to highlight similarities and differences in respect of the two species, this discussion is organised by characteristic rather than species. Where the situation was identical or very similar in both species the account highlights any differences. Where the situation was very different in the two species, separate accounts are given. A summary of the similarities of, and the differences between, the two species, is given at the end of the section in order that their colonies can be seen as entities.

7.2.2 Autozooids, internodes, bifurcations, 'stems' and 'branches', complete and incomplete internodes, 'stem sequences' and colony structure and form

7.2.2.1 INTRODUCTION

A central question underlying this study was does the structure and form of a colony primarily result from the configuration of available space, or is there an intrinsic structure and form which may be modified by any such constraint?

The structure of the colony results from the spatial arrangement of different length internodes, of 'stems' and branches', and complete and incomplete internodes; their arrangement in 'stem sequences'; and their characteristics and spatial distribution within the colony. Is order apparent in these characteristics? If so, beyond a description of the totality of these spatial relationships there are clearly questions regarding the mechanism(s) by which they occur; and why they might be beneficial to the colony. There is probably interaction of a genetically determined astogenetic pattern, and environmental factors which may modify it.

7.2.2.2 AUTOZOOIDS WITHIN INTERNODES, AND BIFURCATIONS

The length of a complete internode is determined by which autozooid within it produces not only another distally, but also one distolaterally, the apical autozooid. It

would have been very useful to know whether or not an incomplete internode had ceased to grow, or was still growing when the colony was collected. It was not possible to reliably determine this for all incomplete internodes, but it is probable, at least for the large colony of the detailed study of *S. reptans,* given the arrangement of internodes within 'aggregations', that only a minority were still growing. If internode lengths, and/or, genuinely incomplete internodes exhibit any pattern of occurrence within a colony, this suggests organisation and presumably a degree of colonial control.

The most notable feature of internode lengths for both species was that, except for perhaps the very first internode (in *S. reptans* only), all consisted of an odd-number of autozooids (Tables 5.8 and 6.3). Lutaud (1953) observed this and was clearly aware of the asymmetric nature of bifurcations (each of which is a mirror image of its predecessor) and the absolutely consistent branching pattern (Figure 5.3) related to it.

Wass (1977) looked at branching patterns in the Vittaticellidae and formulated three laws of branching, only one of which (the most fundamental) occurred throughout the family. Autozooids within internodes are arranged very differently from the species of this study and do not appear to inevitably result in asymmetric bifurcations of opposite hands alternating. Nevertheless they do, and the branching pattern (as Wass' first law) is identical to the one described by Lutaud (1953) and observed here in both species. Constantly reversed handing of asymmetric bifurcations is probably widespread; it occurs, for example, in the cheilostome genera *Eucratea* and *Bugula* (Hayward and Ryland, 1998), in some biserial cellularines, and in some crisiids (Harmer, 1891).

Clearly internodes produce an apical autozooid before they bifurcate, and it is difficult not to see the consistent branching pattern as resulting from the consistent production of internodes containing an odd-number of autozooids and the asymmetrical bifurcations. It is significant that, for *S. reptans,* the only internode that may consist of an even number of autozooids is the first, where there is no previous bifurcation 'handing' to be reversed.

Both species contained a range of complete internode lengths although the two species were very different in respect of those lengths that occurred most frequently. In *S. reptans,* internodes of seven autozooids were most numerous and those of seven or five, constituted > 90% of the total, with a few having 9 or 11 (Table 5.8). In contrast, in *T. inopinata,* internodes of five were the most numerous, and those of three or five constituted 80% of the total, with a few of 7, 9 or 11 (Table 6.3).

In the colony of *S. reptans* the different length internodes exhibited a complex if ill-defined pattern, in which the longer the length of internode the more distal, generation-wise, was its first appearance and the more proximal its disappearance (Table 5.7). Although the colony of *T. inopinata* was less developed than that of *S. reptans,* a similar pattern of occurrence was apparent. It differed in that the first five generations of internodes were all of the shortest length, three autozooids (Table 6.2). There would appear to be some form of control, presumably colonial, here. Five generations of very short internodes, maximising the number of growing points in the early stages of growth, would seem very much in keeping with the opportunistic character of the species (Occhipinti Ambrogi, 1991; Occhipinti Ambrogi and d'Hondt, 1994).

The vertical pattern of the occurrence of the various length internodes referred to above, probably largely results from their arrangement within 'stem sequences', and the arrangement of 'stem sequences' of various lengths within the colony. These aspects will be discussed in Sections 7.2.2.5 and 7.2.2.6.

Continuous exponential increase in the number of internodes, generation on generation, is clearly impossible in a finite space, and some internodes do not bifurcate. In the colonies of both species investigated some 40% of internodes were incomplete (Tables 5.8 and 6.3). Their spatial disposition exhibited no real small-scale pattern.

7.2.2.3 'STEM' AND 'BRANCH' INTERNODES

'Stem' and 'branch' internodes were distinguished purely in relation to the bifurcation which gave rise to them. There was no reason to expect to find, since each bifurcation gives rise to one of each, any asymmetry in their occurrence.

For the colony of *S. reptans* studied in detail there were ~ 20% more 'stems' than 'branches' (Tables 5.9 and 5.10). In the *T. inopinata* colony the situation was very different, for complete internodes there were 50% more 'stems' than 'branches' (Table 6.4) whilst for incomplete internodes there were 50% more 'branches' than 'stems' (Table 6.5). There was a very pronounced tendency for 'stems' to bifurcate and for 'branches' not to. These figures and the differences between the two species were initially very unexpected and suggested that 'stems' and 'branches' might, in some respect, fulfil different functions.

For *S. reptans* the numbers of 'stems' and 'branches', by internode length, exhibited asymmetries, the longer an internode the more likely it was to be a 'stem' (Table 5.9). For *T. inopinata* although the overall 'stem' to 'branch' ratio was 60:40, none of the three lengths present in quantity (three, five or seven autozooids) were close to this. For these internodes, the ratios were 40:60; 85:15; and 50:50 respectively, and for the few longer internodes the ratio was 25:75. Longer internodes tended to be 'branches' (Table 6.4). Do these asymmetries suggest, perhaps, some cryptic arrangement of internode lengths within the colony?

7.2.2.4 'STEM SEQUENCES'

Distinguishing between 'stem' and 'branch' internodes led to the concept of 'stem sequences', a level of organisation between the internode and the colony. In the preliminary study of *S. reptans,* whilst main 'stem sequences' were constituted overwhelmingly of internodes of five autozooids, internodes of seven were probably the most numerous outside them. This posed the questions:-

- If there was a main 'stem sequence' which differed from the colony overall in respect of its internode composition, were there perhaps other 'stem sequences' which exhibited a similar character?
- Could there be other characteristics in which main, or indeed any other, 'stem sequence' differed from the rest of the colony?
- If there were a number of 'stem sequences' with particular characteristics, did other 'stem sequences' exhibit any particular relationship to them?

Again any pattern in respect of internode or 'stem sequence' length surely indicates some form of colonial control.

7.2.2.5. INTERNODES WITHIN 'STEM SEQUENCES'

Two characteristics of the arrangement of internodes within 'stem sequences' were apparent in both species:-

- Long 'stem sequences' were composed virtually entirely of short internodes (Tables 5.14 and 6.9).

(A suggestion of the existence of long 'stem sequences' composed of short internodes can be found in Harmer (1923). In respect of 'branches' in *Tricellaria* he wrote, "internodes commonly constituted by three zooecia, at least in the main stems").

- Within a 'stem sequence', long internodes rarely occurred beyond the more proximal positions within it (Tables 5.13 and 6.8).

Regarding the lengths of internodes within 'stem sequences' the situation was very similar in both species, allowing for the different proportions of the various lengths between the two. For *S. reptans,* in the main 'stem sequences' of the preliminary, and the 10 longest 'stem sequences' of the detailed study, 98% and 93% respectively of internodes were of five autozooids or less, and none were of more than seven (Tables 5.1 and 5.14). Within the colonies internodes of seven autozooids were the most numerous and longer internodes also occurred (Table 5.15).

Although the internode lengths which occurred most frequently in *T. inopinata* were different, shorter than in *S. reptans,* a very similar pattern was apparent in respect of both of the characteristics described above. Internodes of three autozooids were more numerous, however, and long 'stem sequences' were of internodes of three and five (Tables 6.9 and 6.10).

For the colony of *S. reptans* analysis showed that within all 'stem sequences' (Table 5.13) internodes of >5 autozooids were very largely restricted to the more proximal,

and internodes of >7, to the most proximal positions of internodes. The situation was very similar for the colony of *T. inopinata* (Table 6.8).

The fact that in both species long 'stem sequences' were constituted overwhelmingly of short internodes and that none of the longest lengths were present, will be discussed below in connection with the structure and form of a colony. It is difficult to see what disadvantage, in itself, could result from long internodes occurring in other than proximal positions within a 'stem sequence'. It is possible, given that long internodes generally signalled the termination of a 'stem sequence', that the former are related to how the latter occurs.

The first internode of each 'stem sequence' is a 'branch' and all subsequent internodes within them are 'stems'. The relative numbers of 'stems' and 'branches' results simply from the relative numbers of the various length 'stem sequences'. Similarly with complete and incomplete 'stems' and 'branches', the longer the 'stem sequences' the more 'stem' internodes will tend to be complete, and the shorter the 'stem sequences' the greater the probability that 'stem', and if very short, 'branch', internodes will be incomplete.

The very different proportions of complete and incomplete 'stems' and 'branches' in the colonies of the two species investigated in detail may result from intrinsic differences in the numbers of 'stem sequence' of various lengths in the two species, or perhaps from different stages of growth of the two colonies. The proportion of incomplete internodes essentially increases generation on generation (Tables 5.6 and 6.1). As stated earlier it was not possible to differentiate terminally incomplete internodes from those which were still growing when the colony was collected. Nevertheless the proportion of total internodes that were incomplete was almost identical, at 40% in the colonies of both species.

In *S. reptans* only internodes of >7 autozooids exhibited a disproportionate 'stem'/ 'branch' occurrence. They were concentrated in the most proximal positions within a 'stem sequence', but more frequently in the second rather than the first (Table 5.13). In *T. inopinata* all internode lengths, with the exception of internodes of five, declined rapidly in frequency of occurrence moving distally along a 'stem sequence'.

Those of five occurred twice as frequently in the second position than the first. (All first position internodes are 'branches', and all second position 'stems'). Internodes of five dominated the more distal positions within 'stem sequences', together with a minority of three autozooids (Table 6.8).

The figures relating internode lengths to position within a 'stem sequence' (Tables 5.13 and 6.8) rearrange the figures for complete 'stems' and 'branches' by internode length (Tables 5.9 and 6.4). Do the former explain the asymmetries so apparent in the latter? There is clearly some intrinsic pattern of internode lengths in relation to internode generation, especially so for *T. inopinata.* In the colony of the detailed study only internodes of three occurred in the first five generations, and then the situation changed very quickly over subsequent generations (see Figure 6.2). Nevertheless it is difficult not to feel that the numbers and spatial distribution of the various lengths of 'stems' and 'branches' result in large part from the essentially consistent pattern of their occurrence in 'stem sequences'.

7.2.2.6 INTERNODES AND 'STEM SEQUENCES' WITHIN THE COLONY

Brief mention was made in Section 7.2.2.2 to the distribution of the various lengths of complete internodes in relation to internode generation. Their distribution in relation to the 'stem sequences' in which they occurred, and their spatial distribution within the colony, are probably more fundamental in this respect.

Clearly a pattern of occurrence of different length internodes related to the position of an internode within its 'stem sequence' will not result in a general vertical or lateral pattern within the colony. As a result the most visually apparent lateral feature was the existence of a limited number of long 'stem sequences' of short internodes. The changes in the proportions of the various length internodes with internode generation (Section 7.2.2.2) result, in central and distal generations, from the changing proportion of internodes within a generation that are proximal internodes within a new 'stem sequence', or more distal in ones established earlier.

The initial exponential growth in the number of internodes could not continue indefinitely. The questions were, at what stage and at what rate, did slowing occur, and was there any pattern to the spatial arrangement of internodes and 'stem sequences' which resulted?

This section is very largely based on the colonies studied in detail, but the salient characteristics were also apparent in the other studies and in all of the material investigated of both species.

For *Scrupocellaria reptans* the changes in the numbers of internodes over the generations (Table 5.6) result from, in two-dimensions, the lanceolate shape of the 'aggregations' of internodes which developed in central and distal regions of the colony, in association with long 'stem sequences', discussed below. The potential for exponential growth was exploited only in the first five generations. For the next 10 generations the number of internodes increased modestly, at a decreasing rate, and then declined over the final 10.

Laterally also, a pattern was apparent, if not rigidly defined (see Figure 5.4). Beyond the proximal region of the colony where all possible internodes did develop, there was a large scale 'thinning out' which increased in magnitude, generation on generation. As a result, internodes occurred generation by generation in a small number of narrow groups with increasingly large spaces between them.

For *Tricellaria inopinata*, although the colony of the detailed study was less fully developed, the situation was very similar (Figure 6.3) as the examination of other colonies confirmed.

The spatial arrangement of internodes, both their numbers and lengths within a colony, has to be seen in the context of the lengths and spatial arrangement of its 'stem sequences'.

Three characteristics of the arrangement of 'stem sequences' within a colony, were apparent in both species:-

- Colonies consisted of a very limited number of long 'stem sequences' and a much larger number which were short or very short (Tables 5.12 and 6.7).
- When exponential growth in the number of internodes yielded to limitations of space, growth occurred only in discrete 'aggregations' of internodes, each in association with a single long 'stem sequence' (Figures 5.4 and 6.3).
- Growth within each 'aggregation' of internodes, within short 'stem sequences', was restricted laterally to a vertical lanceolate shaped band (Figures 5.5 and 6.4).

The situation was very similar in both species and clearly fundamental to their overall structure.

(Wass (1977) in respect of *Orthoscuticella lorica* (referred to above in relation to its branching pattern) referred to primary, secondary and tertiary branches but made no reference to their lengths. Busk (1852) figured *O. lorica* (as *Catenicella lorica*), and clearly shows what I would describe as a small number of very long 'stem sequences').

Further evidence apparently supporting the significance of 'stem sequences' of different lengths was apparent when, in the colony of *T. inopinata,* the level of occurrence of lateral avicularia and ovicells was related to the lengths of 'stem sequences'. The occurrence of lateral avicularia was positively correlated with 'stem sequence' length (Table 6.24), whilst the occurrence of ovicells was the converse (Table 6.34).

I shall describe the situation in respect of *S. reptans* and indicate for *T. inopinata* those minor respects in which it differed.

For *S. reptans,* in the colony of the detailed study there was a very small number of very long, and a very large number of short or very short, 'stem sequences' (Table 5.12). All of the long 'stem sequences' originated in the first six generations of internodes, although short 'stem sequences' also originated in generations four to six. (One very long 'stem sequence', in a colony of *T. inopinata,* used in a supplementary study, originated in the eighth generation). There was, therefore, no absolute

relationship between internode generation and whether or not a 'stem sequence' became long. From generation seven on, and the colony reached generation 28, 'stem sequences' were overwhelmingly (94%) of four internodes or less.

In *S. reptans,* the limited number of very long 'stem sequences' and, beyond the more proximal generations of internodes, the very strongly clumped distribution of internodes in association with them (Figure 5.4) resulted, in the central and distal regions of the colony, in discrete zones of extensive vertical growth. Each of these, in two dimensions, was a lanceolate 'aggregation' of internodes with a long 'stem sequence' at its centre, developing in a shallowly sinusoidal manner essentially maintaining a constant direction of growth (Figure 5.5). A very small minority of internodes formed discrete thin 'arms' which diverged widely from this central core, and these may have been part of the structure or merely aberrations resulting from a local failure of 'the system'. The situation was identical in *T. inopinata* (Figure 6.4).

The changes in the numbers of internodes over the generations referred to above in *S. reptans* result from the shape of these 'aggregations'. The numbers produced would seem to be the numbers required to form that number of 'aggregations' of that shape rather than internodes ceasing to grow because of mutual interference. In *T. inopinata,* the frequently much more incurved 'aggregations' were perhaps more likely to lead to internode interference and inhibition.

How did the internodes within 'stem sequences', within these 'aggregations', develop in two dimensions into this lanceolate shape? If all possible internodes developed, such an 'aggregation' would form an inverted triangular shape which increased in width exponentially, generation on generation. Whilst it is not surprising that this did not occur, what did was a very considerable modification. For *S. reptans*, as referred to above, it presumably results from a spatially organised pattern of internode and 'stem sequence' termination and/or suppression, although I have been unable to discern any small-scale pattern by which the large scale one occurred. This may also be the case for *T. inopinata*, but mutual interference and inhibition of internodes may occur in this species.

As was noted earlier, the length of an internode is determined by which autozooid within it buds the apical autozooid. Although the mechanism activating this is not known, the occurrence of sequences of particular length internodes, within the long 'stem sequences', suggests some form of control, presumably colonial. Is there a control mechanism that determines which autozooid buds an apical autozooid (and hence the length of an internode) and which triggers its occurrence? If that should be the case is it such a large step for no such 'instruction' to be given, for the internode not to bifurcate, and the 'stem sequence' to terminate? In *T. inopinata* especially, very long incomplete internodes existed which anthropomorphically appeared positively hedonistic!

In *S. reptans* the very long 'stem sequences' were composed almost entirely of internodes of five autozooids, and in *T. inopinata* of internodes of three and five autozooids. For both, the combination of their very long length, together with a unique internode constitution, points to their being a primary feature of the structure of the colony.

7.2.2.7 COLONY STRUCTURE AND FORM

Because the two species exhibited a very similar structure I shall discuss that of *S. reptans* and then identify in what respects *T. inopinata* differed.

The arrangement of internodes and 'stem sequences' described above has to be seen in relation to the resultant three-dimensional structure of the colony. In Section 7.2.2.5 I described the existence of a limited number of largely discrete areas of growth, each of which, centred on a long 'stem sequence', was long but laterally limited. Each 'aggregation' of internodes developing from a long 'stem sequence' is lanceolate in two dimensions. As a result of the angulation of internodes in relation to one another at bifurcations, and to a lesser degree the lengthwise concavity of the longer internodes, 'aggregations' are incurved laterally and distally. In three dimensions 'aggregations' are essentially fusiform, if incompletely so. The colony is an incomplete circle of incurved, incomplete 'flasks', which develop from a vertically limited (proximal to distal), laterally continuous, proximal region.

Colonies of *S. reptans* not constrained by irregularities of available space may still occur in a range of forms from a broad, shallow, cup-like shape, to a much narrower but taller form. This aspect is much influenced by the extensiveness of the substrate. Procumbent colonies on more extensive substrata, less constrained laterally, may perhaps have a greater number of long 'stem sequences' and/or laterally more extensive and less incurved 'aggregations' developing from them, than colonies of a more erect habit.

There is a tendency for colonies of *T. inopinata,* which is very similar in overall structure to *S. reptans,* to differ somewhat in form. Colonies may develop below the horizontal plane of limited substrata and envelop it to a greater or lesser extent, which I have not seen in *S. reptans.* The overall colony form of *T. inopinata,* with very incurved 'aggregations', often like partly clenched fists, is also often much denser.

Before considering why the characteristics described above might be desirable it is necessary to consider the nature of arborescent sessile colonies, the problems they face, and possible solutions to them.

7.2.2.7.1 Characteristics of arborescent sessile colonies, the problems they face, and possible solutions to them

Most sessile animals (and plants) in an aquatic (or indeed terrestrial) environment have an essentially radial symmetry, with a generally convex upper surface (Wainwright et al., 1976). Both of these characteristics, for sessile marine colonies, would seem to be desirable in that they reduce the level of drag experienced by a colony. An essentially radial symmetry, especially for colonies with a single point of attachment, also facilitates an increase in the extent of the feeding surface area.

Sessile aquatic animals are oriented to the direction of water flow, anisotropy (Wainwright et al., 1976). In environments in which food is brought in currents essentially from one direction, planar fan-shaped colonies are the norm but more commonly, in less constant conditions, globular, tree or cone shaped colonies predominate (McKinney, 1981; Ryland and Warner, 1986). Apart from any

irregularities resulting from the form of their substrate, arborescent colonies grow into a three-dimensional volume within the water column. Ecologically a branched modular growth form represents a way to exploit spatially distributed resources (Waller and Steingraeber, 1985). It has been said, regarding the evolution of such arborescent forms, that they can be considered as sheets which have repeatedly subdivided (Harmer, 1923). Unilaminate forms are composed of closely spaced narrow branches with each branch, and the spaces between them, generally 1mm or less. In bryozoans in which zooidal apertures are close together their polypides may co-operate to produce feeding currents which are more effective than those produced by zooids functioning independently (McKinney and Jackson, 1989). Bryozoans constituted of narrow unilaminate branches have zooids orientated towards only one side. When they feed, their obliquely truncate lophophores generate a current that is drawn towards, and then passes by, the branches (Cook, 1977; Winston, 1979).

Erect growth form is generally considered to confer three main advantages relative to an encrusting habit: (Cheetham, 1971; Jackson, 1979).

- Reduced competition from encrusting competitors (Sebens, 1982) and predators on the substratum.
- Increased tissue area for feeding and reproduction per unit area of substratum (McKinney and Jackson, 1989).
- Increased access to food within the water column. At the interface between a solid surface and a moving fluid the velocity of the fluid is zero ('no slip') and increases gradually with increasing distance above it, the boundary layer (Vogel, 1981). Increased height above the substratum therefore brings access to faster rates of flow (Ryland and Warner, 1986).

Whilst the above are probably all true, although not demonstrated experimentally (McKinney and Jackson, 1989) there are also costs to the adoption of an erect growth form:-

- Such colonies are very dependent on their ability to resist damage by water movement, especially those colonies with a single point of attachment (Jackson, 1979). Whether the threat of damage is countered by

reinforcement or the production of flexible elements (Wainwright et al., 1976) an energetic cost is incurred (Denny, 1988).

- Erect colonies are more susceptible to browsing (McKinney and Jackson, 1989).

The structure and form of erect colonies, therefore needs:-

- Firstly, to be such that the possibility of damage by water movement is minimised.
- Secondly, that it exploits the potential advantage of being within the water column by the possession of an extensive area of zooids, so arranged to enhance the feeding ability of the colony.

Whilst increases in the extent of the surface area increase feeding capability, they also heighten the risk of damage due to the action of water movement, raising the level of drag acting on the colony. Increases in colony height and cross-sectional area (both relevant in the two species considered here) increase the level of this (McKinney and Jackson, 1989).

Erect bryozoan colonies are either rigid, deriving their strength from enhanced calcification of the entire structure, or flexible, in which all or certain elements are flexible and allow the colony to bend (Wainwright et al., 1976). In areas of vigorous water movement most erect bryozoans are of the latter type, with rigid forms occurring more frequently at increased depth in quieter waters (Schopf, 1969).

One other aspect for those species which are potentially capable of exponential increase in the number of growing points, is the inevitable constraint of finite space. As colonies grow there is a need for the number of points of growth to be limited.

7.2.2.7.2 How do the characteristics of the structure of a colony described earlier accord with the general considerations detailed above?

(Beyond the arrangement of internodes within the colony, both *S. reptans* and *T. inopinata* have narrow calcified branches separated by flexible chitinous joints, and

are attached to the substratum by numerous flexible rhizoids; adaptations to life in moving water).

The production of a sub-circular arrangement of a limited number of 'aggregations' of internodes each of which is incurved laterally and distally results in the overall form of a colony being essentially radially symmetrical. In more upright colonies there is also a convex upper surface. This structure and form is achieved by an arrangement of unilaminar internodes which bifurcate at intervals, and its three-dimensional nature is largely achieved by virtue of the complex angulation between them.

The limited number of distally discrete 'aggregations' of internodes, their limited lateral extent and the increasing distance (proceeding distally) between them, would appear to systematically control the number and spatial distribution, within a generation, of internodes produced, generation on generation.

The fact that each 'aggregation' is centred on a long 'stem sequence' results in a constant direction of growth. In a colony form in which distally, growth occurs on a limited number of narrow fronts, a constant direction of growth of their central axis is surely advantageous. This idea is supported by the fact that when certain long 'stem sequences' came to an end (perhaps broken) the constant direction of growth was often maintained for several generations, by internodes which developed from internodes laterally adjacent to the distalmost of the long 'stem sequence' (Figure 5.4).

The form of a colony can perhaps be envisaged as if a large circular tablecloth was placed on a smaller circular table, and then inverted. A colony is a series of branches not a continuous sheet, but the surface area of the frontal feeding face of autozooids, within internodes, is clearly very extensive, with only narrow spaces between them. The two dimensional shape of 'aggregations', the angulation between internodes, and the lengthwise concavity of the longer forms, results in their essentially fusiform morphology. This in turn greatly increases the surface area of feeding lophophores and may well enhance the filter feeding process. The essentially sub-circular form of the colony and its 'aggregations', with lophophores being everted into their interior,

results in a reduction in the space between them and also in similar numbers of feeding zooids facing in every direction. Spiral forms such as certain species of *Bugula* (and many hydroids, Cornelius, 1995)) achieve a similar result.

The above is probably an over-simplification. At each bifurcation, because each 'branch' is partially inturned towards its companion 'stem' internode, there may well be still smaller sub-circular forms, within each internode generation, within 'aggregations', each consisting of a series of four internodes. Such an arrangement further increases surface area and may well also increase feeding efficiency. Winston (1979) looking at feeding currents in relation to colony morphology made the following observations in respect of *S. diegensis.* 'The expanded lophophores are directed towards those of the adjacent internode thus occupying the space between the two'. "Viewed from the distal end, the lophophore-bearing surfaces of the colony form semi-circles rather than planar fans, yet the water current passes only once through the colony".

The sub-circular nature of 'aggregations', and perhaps their possession of laterally projecting 'arms', may well, especially for the more plume-shaped colonies, act to keep them separate within the water column.

Long 'stem sequences' constituted of the shortest length internodes to occur in quantity may be advantageous in two respects. Firstly there may be a biomechanical aspect to this, in that it maximises the number of strong flexible joints between internodes, probably desirable in very long sequences of internodes. Secondly it maximizes the number of 'branch' internodes, and thus the number of new 'stem sequences' to which it gives rise. Given that growth only occurs on a limited number of fronts this would seem desirable, the limitations of space being lateral rather than vertical.

Given the overall consistency of the pattern of occurrence and spatial arrangement of internodes within 'stem sequences', the various lengths of these, their arrangement in 'aggregations', and their distribution within the colony, it is difficult not to see them as important elements of the means by which the structure and form of the colony are achieved. The overall structure of a colony results from a combination of the

consistent branching pattern, a large-scale pattern of internode suppression and 'stem sequence' termination, and the angulation between, and the concavity of, internodes. (The three-dimensional structure of the colony would be very different if any of these characteristics did not occur as they do). The consistency of occurrence of these characteristics points to colonial control and suggests a considerable degree of colonial coordination.

7.2.2.7.3 How widespread is the occurrence of this colony structure and form?

It was shown in Chapter 2 (Section 2.4) that for *S. reptans,* damage and partial mortality occurred frequently and significantly. (Colony form involves a compromise between the need to reduce the level of drag acting on the colony, and increase the extent of the surface area of feeding zooids, see Section 7.2.2.7.1 above). It is unfortunate that it is not known to what extent damage was the result of predation or of failure to withstand abiotic forces. Susceptibility to such damage, however it occurs, is likely to be greater in a form in which the majority of the colony develops from a limited number of very long 'stem sequences', than where all 'stem sequences' are of moderate length. They are perhaps, all the more vulnerable occurring as they do within the exposed periphery of the colony.

Nevertheless the structure and form of the colony in both *S. reptans* and *T. inopinata* (and I suspect other unilaminar, biserial cellularines) is clearly a successful one, in spite of the frequency and extent of partial mortality demonstrated for *S. reptans*

This suggests perhaps the more widespread occurrence of this structure and form in unilaminar, biserial, arborescent cellularines. This arrangement may not however be characteristic of all such species. A limited amount of material of *S. scruposa* which I have seen is very different, in that I found no evidence of a limited number of long 'stem sequences' of shorter internodes, around which the colony was structurally organised. Internodes found were of up to 17 autozooids; those of seven or nine autozooids predominated, but none shorter than seven were seen.

A possible explanation may lie in the fact that colonies of *S. reptans* and *T. inopinata* appear to occur, however abundantly, as separate colonies, whilst those of *S.*

scruposa, which I have seen, form a continuous 'turf' in which the scope for lateral growth is very circumscribed. It would seem arguable that *S. scruposa* is less advanced than *S. reptans* in that it perhaps neither possesses, nor perhaps needs, the ability to spatially organise its internodes, and does not produce a structured colony form. This presupposes of course, that a structure or lack of it is constant for a species.

In his description of *S. macrorhyncha,* a species very similar to *S. reptans*, Gautier (1962) referred to the distinguishing features between the two, including that the internodes of the former were of 9 – 21, whilst those of the latter were of five or seven autozooids. (I have found internodes of up to 13 autozooids in *S. reptans*). The spatial arrangement of frontal avicularia and ovicells, as described by Prenant and Bobin (1966) (as *S. macrorhynchus*) is identical to that of *S. reptans*, and Zabala and Maluquer (1988) believe their description is of *S. reptans,* although they ignore the difference in internode lengths. *S. macrorhyncha* appears to combine many of the characteristics of *S. reptans* with the internode characteristics of *S. scruposa.* It is doubtful if a species in which all internodes were of nine or more autozooids could have a structure revolving around a limited number of long 'stem sequences' constituted of shorter internodes. Are there (at least) two different colonial morphologies for these unilaminar biserial arborescent cellularines, one as *S. reptans* and *T. inopinata,* and one as *S. scruposa* and perhaps, *S. macrorhyncha?*

7.2.3 Polymorphic heterozooids and ovicells

7.2.3.1 INTRODUCTION

The situation for polymorphs and ovicells was much more complex than that for autozooids, internodes and 'stem sequences' because:-.

- Firstly, the two species differ in many respects.
- Secondly, there were more zooid types involved, parameters to consider, and possible interactions between them.
- Thirdly there was, for one polymorph of *S. reptans,* evidence of inter-colony, and for two, of inter-population variation. (These have not been given much

attention, as whilst they affected the definition of patterns and trends, they did not negate them).

Polymorphs occurred constantly on all autozooids; predictably on certain autozooids; or, on some, with various degrees of unpredictability. For the first of these their distribution must be genetically determined, there is no question of environmental factors, internal or external being involved, and no question of differential effect on the production of other polymorphs or reproductive activity. For the second group, whilst again their distribution must be genetically determined, their presence or absence may well influence the presence or absence of another polymorph or reproductive activity. In neither group is there any variation in their spatial distribution to investigate. For the third group, their distribution may result from various factors, which may well interact with one another, and whose individual importance may vary temporally.

In *S. reptans*, the predictably occurring frontal avicularia, and the unpredictably occurring lateral avicularia, clearly result from control mechanisms of different plasticity. It is also interesting that whilst the level of occurrence of the former is constant that of the latter is very variable. The situation is somewhat similar in respect of vibracula, the axial occurring at a constant level whilst there was inter-population variation in respect of all other vibracula.

It is tempting to assume that constantly occurring polymorphs must be of the greatest utility to the colony. This may well be so, but the ability to respond appropriately to changing circumstances would seem very desirable. In the context of trying to understand why certain polymorphs occurred constantly or predictably, is it conceivable that this has sometimes resulted from genetic assimilation (Waddington, 1953) and that the ability to respond in a more plastic manner, which would be more advantageous, has been lost? I believe the jury is very much still out on genetic assimilation, but Harvell (1994) felt that it could be a factor by virtue of the late time differentiation of germ cells and their redifferentiation in each newly budded zooid.

For unpredictably occurring or morphologically variable polymorphs and ovicells, their numbers and their spatial arrangement within a colony may well result from the

interactions of a variety of factors. Environmental factors may be what Silén (1977) termed internal or colonial, or external. Genetic factors may well affect the threshold level at which the colony responds to external environmental influences.

If environmental factors, either limiting polymorph occurrence or necessary for its initiation, are involved (and these may vary temporally, and perhaps interact) it is unlikely that any observable pattern of occurrence will develop. Conversely, however, any observable pattern within the colony, especially if not simply regional, is likely to have a substantial astogenetic component.

The observed distribution of a polymorph, or ovicells, whilst it may involve an intrinsically spatial element, may also be influenced positively or negatively by the presence or absence of another polymorph or reproductive activity. It may also be affected by its own overall level of occurrence.

Questions concerning why, when and where a polymorph occurs are very complex. They are not answered simply by describing its observed spatial distribution. Such a description, however, is a necessary prerequisite to any attempt to tease out the interaction of the various factors involved.

For *S. reptans,* the mural spines, scuta, frontal avicularia, and axial vibracula, are constantly or predictably occurring polymorphs, but for *T. inopinata,* the variations in morphology of one mural spine, and the scutum, mean that only for the remaining mural spines can the influence of environmental factors, colonial or external, be ruled out.

The discussion regarding the distribution of heterozooids and female reproductive zooids, as evidenced by the presence or absence of ovicells, is far from straightforward. Numerous parameters of their individual spatial occurrence have been recorded in relation to the constituent parts of a colony, on a variety of scales. (Some asymmetries of occurrence may prove to be secondary and simply result from another). The information obtained is more usefully considered from the aspect of differential production by a region, or constituent element of the colony, on a variety

of scales, but references within the literature are invariably related to 'the polymorph'. The discussion will therefore approach the results from both perspectives. It is also necessary for all polymorphs and ovicells to be considered together in respect of these, and this will include possible associations, positive and negative, between them. Polymorphs with no variation in their level of occurrence need to be included for the picture to be complete. The aim of the study was to look at a colony in its entirety.

I shall therefore sequence this discussion as follows:-

- Firstly, the observed spatial distribution of the various polymorphs and ovicells.
- Secondly, the various apparent associations, positive and negative, between polymorphs or between a polymorph and ovicells.
- Thirdly, the affect that variation in the level of occurrence may have on the spatial distribution of a polymorph.
- Fourthly, an analysis from the 'viewpoint' of the colony and its constituent parts.

7.2.3.2 THE OBSERVED SPATIAL DISTRIBUTION OF POLYMORPHS AND OVICELLS WITHIN A COLONY

7.2.3.2.1 Spines

For both species, apart from the ancestrula which had its own particular arrangement, all autozooids had mural spines, the precise arrangement of which was related to the position of the autozooid within the internode. For *S. reptans* there was no variation of form in respect of the mural spines. In *T. inopinata* the most proximal of the external spines was sometimes bifid. This variation is discussed in Section 7.2.3.5.1.

7.2.3.2.2 Scuta

For both species all autozooids except the ancestrula had a scutum. In *S. reptans* this was always a cervicorn structure, but in *T. inopinata* there was much variation in size and form. This is discussed in Section 7.2.3.5.1.

7.2.3.2.3 Frontal avicularia

No frontal avicularia are present in *T. inopinata.*

In *S. reptans*, excluding the first internode of the colony, large frontal avicularia occurred constantly on all odd-numbered autozooids except No. 1, and were absent from all even-numbered autozooids. Their absence from the first internode of a colony may be simply astogenetic or ascribed to very close arrangement of the autozooids there. Their absence from all No. 1 autozooids was almost certainly due to the very short gymnocyst, which is bisected by the uncalcified joint between internodes. (See Section 7.2.3.5.2 re the occurrence of frontal avicularia and ovicells in *S. reptans*).

The absolutely predictable occurrence of a polymorph indicates that their production must be genetically controlled, albeit in a more sophisticated fashion than where the polymorph is produced on all autozooids. With such a pattern of occurrence, there was clearly no colony-wide aspect to their spatial distribution. They occurred, however, only in the external series of autozooids in both of the internodes resulting from a bifurcation. Why were they always produced on those particular autozooids? It may be another instance of the dichotomy between the two series of autozooids within an internode. It may involve positive or negative correlations between polymorphs or polymorphs and ovicells. Both of these will be discussed below. (Sections 7.2.3.5.1 and 7.2.3.3 respectively).

7.2.3.2.4 Lateral avicularia

Lateral avicularia occurrence in both species was unpredictable. In *S. reptans,* for which material from three populations was examined, there was clear evidence that overall level of occurrence differed between colonies within the Musselwick population, and substantially so between it and the Swanage and Bay Fine populations. The level of occurrence was 3x higher at Swanage, and 5x at Bay Fine, in a sub-littoral population. In respect of the latter, the higher level of occurrence may be related to a generally higher level of biological interaction in such a habitat.

There was often evidence of a clumped distribution, and this may support the idea that they occurred in response to an environmental stimulus, and perhaps that a single avicularium was rarely a sufficient response to it.

In neither species were these avicularia monomorphic. In *S. reptans* most were very small and a few much larger. In *T. inopinata* there appeared to be a continuous range of sizes and virtually all were larger than the small forms in *S. reptans.* The situation in *S. reptans* would seem to be in line with that in many species in which, if both frontal and lateral avicularia are present, one is invariably poorly developed (Harmer, 1923). Whether this is, as Harmer speculated, because one form is declining due to the well developed presence of the other is debateable.

In *S. reptans* the level of overall occurrence of lateral avicularia was very variable; there is no single level of occurrence for the species. Their occurrence at Swanage was approximately three times that at Musselwick and patterns of distribution clearly reflected this. Occurrence of lateral avicularia for *T. inopinata* was investigated in the detailed study and in two additional colonies from the same population. Their level of occurrence in all three was similar at ~30%.

The detailed summary of the results in respect of all the parameters of lateral avicularia occurrence for *S. reptans* is in Section 5.5.3.2.2, and for *T. inopinata* in Section 6.4.3.2.1.

In *S. reptans* lateral avicularia did not exhibit any consistent pattern of occurrence in relation to internode generation. In *T. inopinata*, in relation to internode generation, lateral avicularia initially occurred frequently, but this level soon fell abruptly and did not regain its former level. There is some evidence that this pattern is not due to a direct relationship to internode generation but is related to the distribution of ovicells (Section 7.4.3.2.4.)

In *S. reptans* lateral avicularia occurred more frequently within long 'stem sequences' than outside them. In *T. inopinata,* for which more data were available, the longer a

'stem sequence', the higher the level of lateral avicularia occurrence (Section 7.2.3.5.3.)

In both species (except for the most proximal pair of autozooids in the internode in *T. inopinata*, where the opposite was the case) lateral avicularia occurred more frequently on odd-numbered autozooids in the external series. This may be an element of the general dichotomy between the two series of autozooids within an internode (Section 7.2.3.2.11). Why the small number of lateral avicularia on the most proximal pair of autozooids in *T. inopinata* should occur more frequently on the even-numbered autozooid is puzzling.

In both species lateral avicularia occurred more frequently on higher numbered and especially sub-apical autozooids. The latter may be an element of the former or involve an entirely separate factor. The fact that they occurred much more frequently on complete than incomplete internodes suggests the latter.

Lateral avicularia, in both species, occurred more frequently in complete than incomplete internodes, and on 'stems' rather than 'branches'. This apparently linked asymmetry occurred with other polymorphs and will be dealt with in Section 7.2.3.5.2.

In *S. reptans* the infrequent large forms always occurred in the external series of autozooids, generally on the external of the two sub-apical autozooids. In *T. inopinata*, almost invariably 'large' forms occurred on the external of the two sub-apical autozooids and if lateral avicularia were present on both, the one on the external was always the larger.

The concentration, in species in which most lateral avicularia are small, of large forms on sub-apical autozooids was noted by Osburn (1950) in respect of *S. californica.* Lateral avicularia "are rather small, but these are frequently replaced, especially towards the end of branches, by giant avicularia of about the same form."

(There are also references in the literature to very large frontal avicularia on the axillary autozooid in *S. diadema* and *S. obtecta* (Harmer, 1926; Canu and Bassler, 1929; Osburn, 1950; Ryland and Hayward, 1991).

Why large lateral avicularia should be concentrated on the external of the two sub-apical autozooids is unclear. Such large avicularia are likely to be more effective organs of defence against larger intruders than smaller forms, but there is no obvious reason why they should be sited as they are, or why their occurrence is so episodic.

7.2.3.2.5 Vibracula

Vibracula are absent in the genus *Tricellaria.* Their absence may be invoked to explain the far greater occurrence of overgrowth, generally by other bryozoans, in *T. inopinata*, compared with *S. reptans.*

The frequency of vibracula occurrence at Swanage was more than twice the level at Musselwick.

The detailed summary of the results in respect of all the parameters of vibracula occurrence is in Section 5.5.3.2.3.

Vibracula did not exhibit any pattern of occurrence in relation to internode generation, or autozooid position within an internode. They occurred twice as frequently in long 'stem sequences' than outside them. This may be related to the fact that rhizoids, which develop from them, occur more frequently in long 'stem sequences' (see Section 7.4.3.2.6). It may simply be another instance of polymorphs in general occurring more frequently in longer 'stem sequences' (see Section 7.2.3.5.3).

Vibracula occurred much more frequently on even-numbered autozooids in the internal series. It has been generally assumed that the central function of vibracula in arborescent species was the removal of sediment and/or the discouragement of would be settlers by the sweeping movements of the setae (Cook, 1985; Winston, 1991; Barnes, 1994). It is difficult to see why, if this were the case, they should be strongly

concentrated on one side of an internode. This may be an element of the general dichotomy between the two series of autozooids within an internode and will be discussed in Section 7.2.3.2.11. It may be that vibracula occurrence is related to that of ovicells whose occurrence was essentially restricted to that series (see Section 7.2.3.5.3).

Vibracula occurred more frequently on complete than incomplete internodes, and on 'stems' than 'branches'. This apparently linked asymmetry occurred in respect of other polymorphs and will be dealt with in Section 7.2.3.5.2.

7.2.3.2.6 Axial vibracula

One other polymorph which occurs absolutely constantly in *S. reptans* is the axillary vibraculum, which actually develops from the No. 2 autozooid of the stem internode produced at a bifurcation. Species within the genus *Scrupocellaria* are either without such vibracula, have one, or two; and their occurrence, or lack of it, is generally constant within a species (Waters, 1896). *S. jullieni*, however, while typically having two, may lack one or both (Hayward, 1978). It is difficult to see why variation should occur, given the constant arrangement of autozooids at bifurcations within the genus. In species with two such vibracula, they are produced by the No. 2 autozooid in each internode following the bifurcation. It is difficult to see the utility of having two vibracula situated side by side.

7.2.3.2.7 Rhizoids

Both *S. reptans* and *T. inopinata* are attached to the substratum by tubular rhizoids, which originate differently in the two species. In *S. reptans* the rhizoid chambers develop from vibracula and are, therefore, produced throughout the colony regardless of whether or not a rhizoid will develop. No vibracula are present in *T. inopinata* and rhizoids are produced from rhizoid chambers which are not produced in such a profligate manner. This may well influence the pattern of distribution in the two species.

The numbers of rhizoids a colony produces and the size of the area of the colony in which they occur, are inevitably related to the form of the colony and the extensiveness of the substrate. References to rhizoids within the literature generally refer to the fact they are concentrated in the more proximal region of the colony. Whilst this is broadly true, the two species investigated do differ even in this respect. Rhizoids in *S. reptans* are concentrated in the proximal generations of internodes but this zone is often quite extensive and in well-developed colonies, a second, more distal zone of internodes may also develop rhizoids. In *T. inopinata,* rhizoids are much more noticeably concentrated in the early generations of internodes; the zone probably expanding distally as required. Although Robertson (1905) stated that rhizoid chambers without rhizoids did not occur, I have seen them but only on autozooids in internodes immediately distal to those with rhizoids.

To say simply that rhizoids are concentrated proximally implies that there is no horizontal aspect to their distribution. This is not the case. In both species, rhizoids were concentrated horizontally within the internodes of long 'stem sequences', and those laterally adjacent to them. Given the structure of the colony, this is not unexpected, but again suggests a degree of colonial control.

7.2.3.2.8 Ovicells

Ovicell occurrence was substantially different in the two species, and they will be considered separately before comparing them.

Scrupocellaria reptans

The detailed summary of the results in respect of all the parameters of ovicell occurrence is in Section 5.5.3.2.5.

In a fertile, medium sized colony from Swanage, ovicells were absent from the early generations, occurred at a consistently low level for a number of generations and declined in the most distal generations. Colonies clearly need to establish themselves before commencing reproduction. The absence of ovicells from the more distal generations of a colony, which was probably still growing, seems simply to result

from the time delay between the production of an autozooid and the requirement for, and production of, an ovicell.

Ovicells were very largely absent from the long 'stem sequences' (see Section 7.2.3.5.3 below).

In a second colony from Swanage ovicells occurred much more frequently on proximal than sub-apical autozooids. As far as the general absence of ovicells on sub-apical autozooids is concerned, perhaps the involvement of the autozooids distal to them in the bifurcation, with the change in direction of growth that this involves, is a factor. They are produced by such autozooids however, if infrequently.

Some 97% of ovicells were produced on even-numbered autozooids within the internal series, and while this may be one aspect of the dichotomy between the two series of autozooids in an internode, it is probably related to the spatial distribution of frontal avicularia. Both aspects discussed below in Sections 7.2.3.5.1 and 7.2.3.3.2 respectively.

Tricellaria inopinata

The detailed summary of the results in respect of all the parameters of ovicell occurrence is in Section 6.4.3.2.3.

Before discussing the results in respect of the various parameters investigated in the detailed and supplementary studies, it is worth recalling one aspect that was investigated in Chapter 3. Non-ovicellate and ovicellate autozooids generally appear 'different' and their basic dimensions were recorded. The results (Table 3.1) showed that the mean length of non-ovicellate autozooids was ~20% longer than ovicellate. It was noticeable that non-ovicellate and ovicellate autozooids generally occurred in pairs.

It is difficult to see why non-ovicellate autozooids should be larger, but the difference occurs so consistently that it is surely significant. Regarding ovicells in relation to autozooid size in *Tricellaria aculeata*, Hastings (1943) observed, "non-fertile

internodes consist of relatively long, stout zooecia, and are less tapering proximally and less sinuous in outline than those of colonies with shorter fertile internodes".

The frequency of ovicell occurrence was inversely related to the length of the 'stem sequence' it was in. This is discussed in Section 7.2.3.5.3. Although not quantified, it was apparent when viewing colonies that there were often sequences of short internodes, composed of long autozooids without ovicells. The long and very long incomplete internodes, some longer than the longest complete internodes, were generally composed of shorter autozooids, and invariably completely ovicellate.

There appears to be a general organization with shorter internodes, largely without female reproductive autozooids, in long 'stem sequences' and fulfilling a structural or growth role; with other generally longer internodes of ovicellate autozooids, in short 'stem sequences', maximizing the rate of reproduction. If this is the case it suggests remarkable coordination between structural organization and reproductive effort. It is difficult to see why such an arrangement would be desirable, unless autozooids within long 'stem sequences' are also different in other respects. Are they perhaps structurally more resilient? They are certainly generally longer.

The overall level of ovicell occurrence varied between the different colonies, but in all they were absent from proximal generations and sometimes from the most distal. Ovicells were found right to the growing tip in some instances, and this, given the time gap between autozooid production and ovicell requirement, probably indicates that the internode, often long and incomplete, had ceased to grow.

Ovicells occurred more frequently in branch than stem internodes and in incomplete than complete. This apparently linked asymmetry, although the reverse of that occurring in respect of polymorphs, will be dealt with below (Section 7.2.3.5.2).

Within the detailed study, ovicell occurrence in relation to internode length was investigated, and it was clear that the longer the internode the higher the level of ovicell occurrence. They were almost entirely absent from internodes of three autozooids. The investigation into the level of ovicell occurrence in relation to both

autozooid number and autozooid position, revealed that whilst autozooid number was not a factor, autozooid position was. The extent of the difference between the level of occurrence on proximal and sub-apical autozooids varied between the three colonies, but in two, ovicells occurred >10 x more frequently on proximal than sub-apical autozooids. This explains, to some degree, why the rate of ovicell occurrence increases with internode length.

As for *S. reptans,* the general absence of ovicells on sub-apical autozooids is perhaps also related to the involvement of the autozooids distal to them in the bifurcation, with the change in direction of growth that this involves. It may, however, be related here to the relationship between ovicells and lateral avicularia discussed in Section 7.2.3.3.4.

Scrupocellaria reptans and *Tricellaria inopinata* compared

The most obvious difference between the two species is the overall level of ovicell occurrence which, although variable within a species, was probably three to four times higher in *T. inopinata.* This is well known as an opportunistic species (Occhipinti Ambrogi, 1991; Occhipinti Ambrogi and d'Hondt, 1994; Dyrynda et al. 2000) with considerable powers of colonisation, and a high level of fecundity is clearly an important element of this. In *T. inopinata,* ovicells occurred at a virtually identical level in the two series of autozooids within an internode. For *S. reptans,* the situation was very different, ovicells are essentially only produced in the internal of the two series of autozooids, and they are rarely produced by the sub-apical autozooid of this. There are, therefore, severe constraints on the overall level of ovicell occurrence, and hence reproduction. Clearly fecundity is not as important to this species.

7.2.3.3 POSITIVE AND NEGATIVE CORRELATIONS BETWEEN VARIOUS POLYMORPHS, AND BETWEEN POLYMORPHS AND OVICELLS

As with the observed spatial arrangement of polymorphs and ovicells, such correlations, positive or negative, may be secondary, and result from other factors. The presence or absence of one polymorph may be positively or negatively correlated

with that of another, or with female reproductive activity. A positive correlation could have a functional origin; the juxtaposition of vibracula and ovicells in *S. reptans* is perhaps of this nature. A negative correlation could be simply a matter of the available space, as is probably the case of *S. reptans,* where frontal avicularia and ovicells are virtually mutually exclusive. Is it conceivable that two energy-demanding polymorphs, such as the large frontal avicularia and the active vibracula in *S. reptans,* are not ' ideally' produced by a single autozooid?

7.2.3.3.1 Simple and bifid spines and variations in scuta morphology in *Tricellaria inopinata*

Unfortunately, spines and scuta in this species are very easily damaged, and more pristine material than I have seen would have been required for a quantitative investigation. Nevertheless some discussion is necessary.

In contrast to *S. reptans,* in *T. inopinata* there is morphological variation in respect of the proximal external mural spine, which is sometimes bifid, and in the extensiveness or otherwise of the scuta. The scutum originates proximal to the mid point of the internal rim of the frontal membrane, and thus, however extensive, it does not cover the distal end of the frontal membrane. The proximal external spine, which is frequently bifid, is directed more distally than laterally, overarches little of the frontal membrane, and its forked end is generally close to the opening of the ovicell. It is quite possible that these two variations occur in parallel. If the constancy of the situation in *S. reptans* reflects a constant requirement, the two variations in *T. inopinata* referred to above could result from some third characteristic, which causes the level of protection required to vary. Given that all autozooids have, at some stage, a functioning polypide, it is difficult to see what third element other than an embryo could be involved.

Several authors have observed a sometimes-bifid proximal external spine. Harmer (1926) noted, "bifurcate proximal outer spine" in *S. diadema.* Fransen (1986) describing a new species, *S. carmali* noted, "the outer proximal spine is often one time bifurcated", but he made no reference to variation in scuta morphology, or any relationship between the two, or of any relationship to ovicells. Osburn (1950)

describing *S. regularis,* noted that both the proximal external and internal spines were sometimes bifid, but made no further comment. Regarding scuta in *S. diadema* Harmer (1926) noted that scuta were often absent from proximal internodes, and were found "particularly in those which are provided with ovicells". Regarding the same species (though named as *S. annectens*), MacGillivray (1887) noted that proximal parts have no scuta, whilst those on fertile "branches" are large. Gordon (1986) regarding *T. inopinata* (as *T. occidentalis*) felt that scuta were more extensive on ovicellate autozooids but did not refer to the distribution of bifid spines. The condition of my material makes me reluctant to speculate, but ovicellate autozooids with bifid spines were apparent on a limited number of undamaged zooids. Strangely, infertile autozooids and internodes were invariably more damaged than those which were ovicellate. If there was a relationship between the two polymorphs and the production of embryos and ovicells, this would indicate considerable colonial coordination. It must be admitted that incurring additional costs to protect embryos is not a characteristic one would expect to find in such an opportunistic species. There may of course, be no functional basis underlying these variations, but they are not simply ontogenetic or astogenetic in nature.

7.2.3.3.2 Frontal avicularia and ovicells in *Scrupocellaria reptans*

In *S. reptans,* frontal avicularia and ovicells occupy an almost identical position on the gymnocyst proximal to the frontal membrane. Excluding the first internode of the colony, frontal avicularia were found without fail on all odd-numbered autozooids except autozooid No. 1, and on no even-numbered autozooid. Ovicells were not numerous and found almost exclusively on even-numbered autozooids. The two were virtually mutually exclusive. In the rare instances when an ovicell was produced on an odd-numbered autozooid, it was produced in addition to the frontal avicularium, with both reduced in size.

The absolute constancy of occurrence of frontal avicularia, the low level of ovicell production and the fact that they were virtually mutually exclusive, suggests that their patterns of occurrence may well be related, perhaps one being restricted by the other. If this is so, it would seem more likely that it is the spatial distribution of frontal

avicularia which constrains that of ovicells, and therefore of female reproductive autozooids, rather than the converse.

The arrangement of frontal avicularia and ovicells in *S. macrorhyncha,* a species very similar to *S. reptans,* was described by Prenant and Bobin (1966) (as *S. macrorhynchus*) as one frontal avicularium per pair of autozooids, except the one following the bifurcation. (The figure shows frontal avicularia in one series of autozooids, and ovicells in the other). This is identical to the arrangement in *S. reptans.* Zabala and Maluquer (1988) believe Prenant and Bobin's description refers to *S. reptans* and note that their description and figures of that species refer to pedunculate frontal avicularia!).

7.2.3.3.3 Vibracula and ovicells in *Scrupocellaria reptans*

Vibracula were very largely confined to the internal series of autozooids. In the two aggregations from Musselwick, 94% and 90%, and in that from Swanage, 74% of vibracula occurred in this series. Ovicells were even more heavily concentrated in the internal series. It was, therefore, quite likely that vibracula would occur on autozooids which actually produced ovicells. Furthermore, given that both occurred at the proximal end of an autozooid they would be very close together. In all of the material investigated in the quantified studies, each ovicell had a vibraculum sited adjacent to it.

(With reference to pseudopores in the Cyclostomatida, Ryland (1970) referred to the general assumption that the function of these was to permit the passage of dissolved gasses through an otherwise impermeable barrier. He went on to note that in the brood chambers, the incidence of such pseudopores was twice that occurring in the walls of the autozooids, and that this must be to satisfy the high oxygen requirements of the developing embryos).

Cheilostome ovicells do not contain the results of polyembryony but presumably the uncalcified pores in the ectooecium are to facilitate gas exchange and, if this is so, that there is a potential problem. At low Reynolds numbers is it possible that the movement of vibracular setae could result in increased local water movement, and

increase oxygen levels available to embryos developing within ovicells? Vogel (1981) suggested that the beating of cilia [in an unspecified animal and environment] "may augment diffusive exchange by reducing the amount of semi-stagnant water around an animal".

That this is not the sole explanation for their pattern of distribution, if indeed it is an explanation at all, is obvious from the more frequent occurrence of vibracula. Also, at higher levels of vibracula occurrence as in 'supplementary Swanage', where ovicells were still essentially absent from odd-numbered autozooids, limited numbers of vibracula occurred on these. It is also the case that vibracula consistently occurred more frequently within long 'stem sequences' than outside them, whilst for ovicells the converse was the case. This latter point will be discussed further in connection with the relationship between the distribution of vibracula and rhizoids (Section 7.2.3.3.6). The above suggests that a polymorph may well be involved in more than one association, in addition to any pattern of sites of 'preferred' production.

7.2.3.3.4 Lateral avicularia and ovicells in *Tricellaria inopinata*

There was much evidence in virtually all of the parameters investigated, that in *T. inopinata* lateral avicularia and ovicells were strongly negatively correlated. The results were slightly different for the three colonies investigated.

- Lateral avicularia occurred 2.5 – 6.0 times more frequently on non-ovicellate autozooids (i.e. the autozooid producing the embryo, not the ovicell).
- The sudden fall in the level of lateral avicularia occurrence in a particular generation of internodes, coincided exactly with the sudden increase in the level of ovicell production, and after which they became generally restricted to sub-apical autozooids, where ovicells rarely occurred.
- In relation to 'stem sequences', the longer the 'stem sequence' the higher the level of lateral avicularia occurrence, whilst for ovicells the converse was the case.
- In relation to 'stem' and 'branch' internodes, lateral avicularia occurred 50% more frequently in 'stems', whilst ovicells occurred 50% more frequently in 'branches'.

- In relation to complete and incomplete internodes, lateral avicularia occurred five times more frequently in complete, whilst ovicells occurred almost three times more frequently in incomplete, internodes.
- When autozooid position was considered, lateral avicularia occurred three times more frequently on sub-apical autozooids whilst ovicells occurred 12 times more frequently on proximal autozooids.

The above is all the more surprising given that lateral avicularia (in total) and ovicells occurred at almost identical levels in the two series of autozooids within an internode. Nevertheless, ovicellate autozooids tended not to produce lateral avicularia.

A negative correlation between lateral avicularia and ovicell production is a counter intuitive situation, as it is difficult to see how the presence of a lateral avicularium adjacent to the opening of an ovicell could be disadvantageous. In some species frontal avicularia are found only in association with ovicells, e.g. *S. scruposa* (see Ryland, 1965), *S. inermis* (see Ryland and Hayward, 1977). Given the assumption that such avicularia have a defensive function, this would seem a more understandable arrangement. Harmer (1926) noted in *S. diadema* "frontal avicularia rarely wanting, typically present, at least in the fertile branches".

Given the above, is it conceivable that there is a disadvantage in a single autozooid producing both an embryo and a lateral avicularium? Certainly it is not an absolute factor as they do occur together on a minority of autozooids.

Given the extent to which lateral avicularia and ovicells occur on different autozooids, the question arises why do they sometimes occur together? In part of a densely ovicellate colony of *T. inopinata,* the relationship between lateral avicularia and ovicells was investigated in relation to internode generation. The situation changed from one generation to the next. In early and late generations where there were a number of 'free' autozooids, without lateral avicularia or ovicells, 'dual occupation' did not occur. In the intermediate generations, where both ovicells and lateral avicularia were numerous, there were no 'free' autozooids without either a

lateral avicularium or an ovicell, and the two occurred together on a minority of autozooids.

It may well be that the numbers 'required' in certain generations of internode were such that this 'over-ruled' a preference for the two to be produced on separate autozooids. It would seem inevitable in general, given the various requirements of a colony, that compromises have to be made regarding their satisfaction, and that some prioritisation in the form of a hierarchy of requirements operates.

Given that lateral avicularia and ovicells are so strongly negatively correlated it is difficult to imagine that their individual distributions are not related, and that the distribution of one influences that of the other. Lateral avicularia occurrence always declined in the internode generation in which ovicells occurred in number, and they then continued to be produced essentially only on sub-apical autozooids which rarely produced ovicells. This suggests it is more likely that the production of embryos and ovicells constrains that of lateral avicularia, rather than the converse. Temporally lateral avicularia, which are produced very early, are likely to be produced before embryos, and their non-production would have to be 'determined', in anticipation of future embryo production. This would seem to indicate considerable colonial coordination. Given that there are no frontal avicularia and that lateral avicularia are very largely restricted to sub-apical autozooids, is it conceivable that ovicells are not produced on these because lateral avicularia 'need' to be? (As discussed in Section 7.2.3.2.8, ovicells in *S. reptans* also occurred much less frequently on sub-apical autozooids and this may be a widespread phenomenon with a completely different cause).

The above scenario is the converse of that postulated in respect of *S. reptans,* although in that species the avicularia were frontally sited. Given the ecological character of the two species however, the two postulations are not unexpected. *S. reptans* clearly, to judge from its level of fecundity, does not give priority to reproduction. *T. inopinata,* being very much an opportunistic species, as evidenced by its reproductive fecundity, is unlikely to give priority to defence over reproduction. If the two postulations are correct, it follows that not only is there

considerable colonial control, but that it is operating in opposite directions in the two species.

7.2.3.3.5 Lateral avicularia and vibracula in *Scrupocellaria reptans*

In *S. reptans* lateral avicularia occurred more frequently on autozooids without vibracula. Given the very small size of the majority of lateral avicularia it is difficult to envisage any possible reason why there could be any problem for the two to occur together. This may be another aspect of the dichotomy of polymorph and ovicell assemblages between the two series of autozooids within an internode discussed in Section 7.2.3.3.

7.2.3.3.6 Vibracula and rhizoids in *Scrupocellaria reptans*

Because rhizoid chambers develop from vibracula, it is conceivable that vibracula could be produced at certain sites to facilitate the production of a rhizoid chamber, and therefore a rhizoid. There is some evidence for this, in the form of increased numbers of vibracula within proximal internodes of long 'stem sequences'.

7.2.3.4 OCCURRENCE LEVELS OF POLYMORPHS AND OVICELLS

There was much evidence that patterns of distribution, very apparent at low levels of occurrence, persisted in a much less clearly defined way at higher levels. This was so, both in respect of the spatial disposition of apparently 'preferred' sites of production and in relation to apparent correlations, positive or negative, between polymorphs or a polymorph and ovicells. To some degree this is mathematically inevitable, as the level of occurrence increases, the scope for asymmetry is reduced. In all cases, however, the less well-defined pattern observed at high levels of occurrence was exactly that – a somewhat less distinct version of the one that obtained at lower levels of occurrence. This suggests that although these patterns may be modified by the level of occurrence, they are always present and therefore, presumably significant.

In the colony of *S. reptans* from the Musselwick population looked at in detail, vibracula were virtually confined to even-numbered autozooids, or more precisely the internal series of autozooids within an internode. In material from Swanage, the overall level of vibracula occurrence was much higher, and virtually all autozooids in the internal series had vibracula, and they were also produced on a limited number of those in the external. A similar situation in respect of lateral avicularia and ovicells was described in Section 7.2.3.3.4.

Whatever the cause of the variations in levels of occurrence, which are beyond the scope of this study, the significant point is that patterns of occurrence exist despite them.

7.2.3.5 DIFFERENTIAL POLYMORPH AND OVICELL PRODUCTION IN DIFFERENT PARTS OF A COLONY

7.2.3.5.1 Autozooid series within internodes, and perhaps in relation to bifurcations

In *S. reptans* there was a partial or complete dichotomy between the two series of autozooids within an internode in that they gave rise to two very different assemblies of polymorphs and ovicells. Frontal avicularia were restricted to odd-numbered autozooids in the external series and lateral avicularia also occurred at least twice as frequently in this. Vibracula, as described above, at low level of occurrence were very heavily concentrated on even-numbered autozooids in the internal series, and at a higher level of occurrence occurred on virtually all even-numbered autozooids and on a minority of odd-numbered. This in itself shows that other factors may 'override' this apparent pattern of 'preferred' production. Ovicells were virtually restricted to even-numbered autozooids in the internal series.

Why should this be the case and at what scale is the variation occurring? The two internodes produced at a bifurcation are mirror images of one another simply as a result of the configuration of autozooids at the bifurcation. It may be that the two series simply bear two different assemblies of polymorphs and ovicells. It is also possible that this dichotomy relates to the internal/external series of autozooids within the two internodes, and thus to the bifurcation which gave rise to them.

This is difficult to resolve. Positive and negative associations between polymorphs and polymorphs and ovicells, discussed above in Section 7.2.3.3, may also be important. It may be that frontal avicularia and ovicells, which occupy essentially identical sites, necessarily occur in different series; because frontal avicularia consistently occupy the external, ovicells are restricted to the internal. This is perhaps, in turn, the basis for vibracula occurring in the internal series. (In *T. inopinata* ovicells essentially occurred equally in the two rows as did lateral avicularia overall).

Does the assembly of the internal or external series of autozooids differ in its requirements? Does occurrence in one row confer any advantage relative to the other? For vibracula and ovicells, both occurring in internal series, the latter at least would arguably benefit from being in a 'protected' position. Frontal avicularia occurred only in the external series and lateral avicularia were concentrated here. With a presumed defensive function their occurrence concentrated in this way could be considered advantageous.

It is tempting to think of 'external' as more vulnerable and 'internal' as more protected, but these terms relate here to a simple bifurcation. Several elements point, if faintly, to the arrangement possibly being in relation to the bifurcation. Avicularia generally, and large forms particularly, occurred more frequently in the external, and virtually all ovicells in the inner series, rather than conversely. (If frontal avicularia occurred in the internal series I suspect they could also occur on the first autozooid of that series, No. 2). The occurrence of vibracula in the same row as ovicells could perhaps benefit the latter as discussed above. Winston (1979) in respect of *S. diegensis* observed that expanded lophophores were directed towards those of the adjacent internode thus occupying the space between the two. Given the low Reynolds numbers is it possible that the concentration of vibracula in both internal rows, especially on the more proximal autozooids within an internode, could exercise an influence on local water movement and perhaps enhance feeding?

7.2.3.5.2 'Stems' and 'branches' and complete and incomplete internodes

The fact that I could not distinguish between incomplete internodes which were still growing from those which were not, undermines to some extent any figures related to complete and incomplete internodes. I suspect that for the larger colony of *S. reptans* the proportion of terminally incomplete internodes was higher than in the less fully developed colony of *T. inopinata.*

Given that polymorphs generally occur early and ovicells late, in relation to their autozooid of origin, one might anticipate that in the internodes at the ends of 'stem sequences' polymorphs would perhaps not exhibit reduced occurrence whilst ovicells probably would.

It was apparent that several unpredictably occurring polymorphs in both species, and also for ovicells in *T. inopinata* (for which there were more data) exhibited asymmetries of occurrence in respect of stem and branch and complete and incomplete internodes. This is perhaps best investigated initially in *T. inopinata* with only mirror image patterns of occurrence of lateral avicularia and ovicells to consider. Did these asymmetries result from these internode characteristics? They varied considerably in extent and, in general terms:-

- Lateral avicularia occurred 4x more frequently in complete internodes.
- Lateral avicularia occurred 2x more frequently in stem internodes.
- Ovicells occurred 2.5x more frequently in incomplete internodes.
- Ovicells occurred 1.5x more frequently in branch internodes.

In respect of complete and incomplete internodes the results are the converse of what one might expect.

For complete and incomplete internodes the levels of occurrence on proximal and sub-apical autozooids are relevant. Lateral avicularia occurred 6x more frequently on sub-apical (which only occur in complete internodes) than proximal autozooids; whilst ovicells occurred ~12x more frequently on proximal than sub-apical autozooids.

Whilst the different levels of occurrence in both would seem to result in part from their very different levels of occurrence on proximal and sub-apical autozooids, this was not the sole cause. For lateral avicularia, when only proximal autozooids were considered, they still occurred twice as frequently in complete internodes. For ovicells the over-riding factor is that they occurred on a very high proportion of proximal autozooids in incomplete, and a much lower one in complete, internodes. For lateral avicularia and ovicells there would appear to be intrinsic asymmetries of occurrence, in for me, opposite directions to those anticipated. Lateral avicularia are produced less frequently towards the growing edge whilst ovicells are generally produced right up to it. Anthropomorphically the colony may not be 'concerned' with protecting internodes which will develop no further, and is 'intent' on utilising to the full, its reproductive capacity.

For stem and branch internodes there is no obvious reason why any asymmetry should exist, and here, they presumably result from larger scale patterns of lateral avicularia and ovicell occurrence. For lateral avicularia their level of occurrence is inversely related to the numerical position of the internode in which they occur, within the 'stem sequence' (see Table 6.25). (This actually relates to the length of a 'stem sequence' rather than internode position within it). As a result the level of occurrence is lowest where the potential for it is highest, in the branch internode which initiates each 'stem sequence', (more than 50% of possible sites occur in these). They therefore occur more frequently in stems. For ovicells the situation is the reverse (see Table 6.36) the level of occurrence is very high in the initial branch internode and therefore ovicells occur more frequently in branches. There is no obvious reason why polymorph or ovicell production should be different in stems and branches as such. Given this and that the trends within 'stem sequences' are apparent over a number of numerical positions within them, it is probable that this characteristic is primary, but why it should occur is far from clear.

It was noted earlier in the colony of *T. inopinata*, that for complete internodes there were 50% more stems than branches, but for incomplete there were 55% more branches than stems! These initially very surprising figures result simply from the relative numbers of the various lengths of 'stem sequences', as discussed in Section

7.2.2.3, but would seem to facilitate the production of both lateral avicularia and ovicells.

Lateral avicularia and vibracula in *S. reptans* both occurred more frequently in internodes which were stems rather than branches, and complete rather than incomplete. The asymmetry in occurrence was always much more pronounced in respect of complete or incomplete internodes than stems and branches. The degrees of asymmetry were less at Swanage, where levels of occurrence were higher than at Musselwick. At the latter they varied considerably in extent and in general terms: -

- Lateral avicularia occurred 200–300% more frequently in complete internodes.
- Lateral avicularia occurred 22–35% more frequently in stem internodes.
- Vibracula occurred 150–550% more frequently in complete internodes.
- Vibracula occurred 8–20% more frequently in stem internodes.

In respect of complete and incomplete internodes the levels of occurrence on proximal and sub-apical autozooids are relevant. Lateral avicularia occurred 2–3.5 x more frequently on sub-apical (in complete internodes only) than proximal autozooids; whilst vibracula occurred with equal frequency on proximal and sub-apical autozooids.

For lateral avicularia in respect of complete and incomplete internodes, their greater frequency of occurrence on sub-apical autozooids is a factor. However, when only proximal autozooids are taken into account lateral avicularia still occurred twice as frequently in complete than incomplete internodes. This is not due to incompletely developed distalmost autozooids, which were discounted; it was often due to their complete absence from such internodes. It must be remembered that lateral avicularia exhibited an extremely irregular distribution.

Vibracula occurred with equal frequency on proximal and sub-apical autozooids although they occurred much more frequently in complete internodes. The most notable characteristic of their pattern of occurrence was their concentration on autozooid No. 4. Although these occurred in equal numbers in complete and

incomplete internodes, vibracula occurred more than 6x as frequently in those of complete. As with lateral avicularia, vibracula were often completely lacking on incomplete internodes. Again there seems to be an intrinsic asymmetry between complete and incomplete internodes. The remarks above, in respect of *T. inopinata,* regarding the decrease in the level of polymorph production towards the growing branch tips also apply here.

For stem and branch internodes there is again no obvious reason why any asymmetry should exist. Here, it probably results from larger scale patterns of lateral avicularia and vibracula occurrence. The asymmetries for both polymorphs were not that great and would seem to result simply from the fact that both occurred in the first internode of 'stem sequences' at a lower level of occurrence, than in subsequent generations. The numbers here were much smaller than for *T. inopinata,* but I still suspect that the trend related to internode position within a 'stem sequence' is cause rather than effect.

It appeared that for many polymorphs in both species, and also ovicells for *T. inopinata*, that there was, in terms of 'preferred' sites of production, a link between 'complete' and 'stems' and 'incomplete' and 'branches'. From the above it is clear that the elements determining differential production of lateral avicularia and ovicells in *T. inopinata* and lateral avicularia and vibracula in *S. reptans,* in respect of 'complete' and 'incomplete', and 'stems' and 'branches', are quite separate, and that there is no actual linkage.

7.2.3.5.3 'Stem sequences'

A central aspect of the arrangement of internodes within a colony was their structured arrangement in 'stem sequences' within the colony. It was nevertheless a surprise when it appeared that the levels of polymorph and ovicell production were also related to 'stem sequence' length.

The data were more extensive in respect of *T. inopinata*, as lateral avicularia and ovicells were recorded for the entire colony. The level of lateral avicularia occurrence exhibited a positive, almost directly linear relationship to 'stem sequence'

length. Ovicells conversely were strongly negatively correlated with 'stem sequence' length.

(The data in respect of *S. reptans* were less extensive as the polymorphs had only been recorded in limited areas of the colony of the detailed study. Ovicells were also rare in this colony. The levels of occurrence, especially for lateral avicularia, were also lower; nevertheless the level of their occurrence, and that of vibracula, generally increased with increased 'stem sequence' length. Unfortunately no comparable data were available in respect of ovicells, although they occurred infrequently, if at all, in long 'stem sequences').

Why should unpredictably occurring polymorphs occur more frequently the longer the 'stem sequence'; and ovicell occurrence be so heavily concentrated in short 'stem sequences' in *T. inopinata*?

Almost 50% of all ovicells were produced in 'stem sequences' of a single internode, and a further 37% in 'stem sequences' of two or three internodes. Only one ovicell occurred in a 'stem sequence' of >8 internodes, so there was a negative relationship here which cut across the general vertical, by internode generation, pattern of ovicell occurrence within the colony. The distribution of 'stem' sequences' by length and generation of origin (Table 6.7) shows, not surprisingly, that long 'stem sequences' originated in proximal generations and short 'stem sequences', in much larger numbers, in central and distal generations. Their prolific production coincided with that of ovicells. The heavy concentration of ovicells in short 'stem sequences' would appear to result simply from the fact that both occur in number, essentially in the same generations of internodes. Their general absence from internodes of the long 'stem sequences', within these generations, is the interesting feature.

For lateral avicularia, which exhibited a mirror image pattern of distribution, their high level of occurrence in longer 'stem sequences' may well be related to the absence of ovicells. Their low level of occurrence in short 'stem sequences', the majority of which were initiated in the internode generations in which ovicell production was at its peak, also probably results from this (see Section 7.2.3.3.4).

The low level of ovicell production in long 'stem sequences' appears to be a primary characteristic related to 'stem sequence' length. Ovicells are produced at a high level in short 'stem sequences'. The distribution of lateral avicularia in relation all 'stem sequences' results in large part, I suspect, from that of ovicells (see also Section 7.2.3.3.4).

7.2.3.5.4 'Aggregations' of internodes

There was an initial requirement, in regard to investigating the spatial arrangement of unpredictably occurring polymorphs in *S. reptans*, to establish regions of a colony which constituted genuinely representative samples of the whole. The discovery of a limited number of 'aggregations' of internodes associated with long 'stem sequences' probably provided such sub-samples of the mass of the colony, even though they excluded the proximal region. It also seemed possible, that if apparently unpredictably occurring polymorphs exhibited any large-scale pattern of occurrence, it could well be in relation to these 'aggregations'. It has to be said that that no large-scale patterns of occurrence in relation to them were observed.

There was evidence in both species, however, that for all unpredictably occurring polymorphs and ovicells, long 'stem sequences' were 'different' from the rest of the 'aggregation'. The polymorphic heterozooids were produced more frequently, and ovicells much less frequently, if at all, within them. The internodes at the centre of the structure of the colony are very largely without female reproductive zooids.

7.2.3.5.5 The colony

Two intrinsically regional patterns of occurrence in both species, both well known, are the proximal production of rhizoids and the absence of ovicells from that region. These patterns of distribution are not problematical. Rhizoid production is essential in the early generations of internodes to anchor the colony, but progressively less so once it is securely attached. Ovicells are absent in early generations, the colony needing to establish itself before commencing reproduction.

7.2.4 *Scrupocellaria reptans* and *Tricellaria inopinata* compared

Some newly described characteristics of *S. reptans* were also found in *T. inopinata*, confirming their significance if not explaining them. For other aspects the situation was very different in the two species, and this encouraged me to look at the sum of the characteristics of the two species as alternative coherent strategies.

The two species exhibited identical characteristics in respect of the way autozooids were arranged in internodes, internodes bifurcated, and the pattern of branching which resulted. The constitution of the colony in respect of the numbers of the various lengths of 'stem sequences', their internode composition and spatial arrangement were also very similar. The structure of a colony was essentially the same in both species but differed in certain respects. The lengths of internodes were generally shorter in *T. inopinata,* and their pattern of occurrence in relation to internode generation was slightly different with early generations being exclusively of very short internodes. The latter would facilitate rapid growth for an opportunistic species. The proportions of internodes in respect of their length, whether they were stems or branches, or complete and incomplete, were very different in the two species. There may be intrinsic differences here or they may result largely from variation in the stage of development of the two colonies. Although the form of a colony within a species did vary, particularly in relation to the extensiveness of their substrata, the two species typically differed in that colonies of *S. reptans* were generally more open and the 'aggregations' less incurved, than those of *T. inopinata.*

In respect of their polymorphs and ovicells the two species were very different. In general, as was already known, *S. reptans* had the more extensive assembly of polymorphs. In *S. reptans* different assemblies of polymorphs and ovicells occurred in the two different series of autozooids within an internode, possibly in relation to the bifurcation. This was not the case in *T. inopinata.* Interestingly whilst spines and scuta are unvarying in form in *S. reptans,* in *T. inopinata* the situation is more complex in that one of its spines is sometimes bifid, and scuta are of variable morphology. This appears somewhat at odds with its characterisation as an opportunistic invasive species. (It is difficult not to view *S. reptans* as more 'K', and *T. inopinata* as more 'r', selected organisms). For aclonal animals, the concept of r

and K–selected species is perhaps today considered less clearly defined than previously (Wilbur et al., 1974). For clonal species, which typically include in the same animal characteristics attributed to opportunistic and specialist species, the concept tends to break down completely (Jackson and Coates, 1986). In both species avicularia were negatively associated with ovicells and it is very difficult, given their assumed defensive function, to see why this should be. It would also appear that in *S. reptans*, ovicell, and presumably embryo production may be constrained by the occurrence of frontal avicularia. Conversely in *T. inopinata* it may well be embryo and ovicell production which constrains that of lateral avicularia. The absence of vibracula in *T. inopinata* could be invoked to support the idea that they prevented settlement, overgrowth occurring much more frequently than in *S. reptans*. *T. inopinata* was considerably more fecund than *S. reptans*.

7.2.5 Models

The approach adopted in this study has been an empirical one and the overall structure of a colony shown to result from a combination of several quite discrete factors. A consistent branching pattern, a large-scale pattern of internode suppression and 'stem sequence' termination, and the angulation between, and the lengthwise concavity of, internodes, are all essential elements. The relative importance of these is unlikely to be constant throughout the growth of a colony. Modular organisms may have fairly 'simple' rules of growth, but these generally change over an organism's lifetime in response to changing internal, colonial, or external conditions (Waller and Steingraeber, 1985).

Models of branching are constructed for different reasons, with corresponding variations of approach. Essentially a mathematician describes branching patterns in terms of nodes and internodes (Bell, 1986). Models of branching may be qualitative or more frequently, with the use of computer simulations, quantitative. The latter take many forms, they may be non-spatial, incorporating only rules of bifurcation, or spatial, and if the latter, two or three-dimensional. The model may predict a constant developmental pattern, deterministic, or with a certain number of built-in random effects, stochastic. Finally a model may assume that rules remain constant,

stationary, or that they change, perhaps more realistically, non-stationary (Waller and Steingraeber, 1985).

Two-dimensional spatial models of encrusting Bryozoa (Gardiner and Taylor, 1982) are comparatively straightforward. Three-dimensional models (Cheetham and Hayek, 1983) which involved, in addition to bifurcation angle, the angle of twist between successive bifurcations, are considerably more complex.

"A model represents the isolation of certain features of a complex situation so that their mutual relationships can be seen without the distraction of other features of lesser significance" (Mackay, 1975). It has been demonstrated that a small change in the specified angle of bifurcations can produce a considerable change in the structure which results (Gardiner and Taylor, 1982). Omitting an essential element of a branching pattern would clearly have a more substantial impact. Whilst models may be used quite simply to investigate particular aspects of growth, complete modelling of a colony requires, "either the morphology of the organism must be recorded in considerable detail, or the underlying features of its developmental architecture fully appreciated" (Bell, 1986).

For the species of this study the length of internodes and angle of bifurcation may be straightforward. Given the complexity of the angulation and twisting of internodes at bifurcations, that they occurred in several different planes, and that the two internodes produced were very different from one another in this respect, modelling the complete structure would seem very difficult. All of the above ignores what Bell (1986) referred to as "the lynchpin in branching astogeny...the control of new branch initiation". This study may suggest parameters which need to be incorporated into any computer generated model but it has not quantified them. In identifying the repeated occurrence of 'aggregations' of internodes in association with long 'stem sequences', it perhaps suggests a more limited structure which could benefit from such an approach.

7.3 UNANSWERED QUESTIONS AND SUGGESTIONS FOR FURTHER WORK

7.3.1 Unanswered questions

Because I quantified parameters and relationships which generally had not, as far as I am aware, been investigated before, it was always likely that unexpected results would be revealed for which there were no obvious explanations. Unanswered questions include the following:-

- Does the occurrence of long 'stem sequences' composed essentially of short internodes, result from colonial control of internode length?
- Does internode and 'stem sequence' termination result from colonial control of internode length and whether or not a bifurcation should occur?
- What is the advantage of lanceolate (in two dimensions) and fusiform (in three) 'aggregations' of internodes in association with long 'stem sequences'?
- Why are colonies of *T. inopinata* so much denser than those of *S. reptans*?
- Why are colonies of *S. reptans* and *T. inopinata* composed of a limited number of 'aggregations' of internodes and colonies of *S. scruposa* so very different in this respect?
- Why should long internodes be essentially restricted to proximal positions within a 'stem sequence'?
- Why the concentration of large lateral avicularia on external sub-apical autozooids?
- Why, in *S. reptans*, is the assembly of polymorphs and ovicells so different in the two series of autozooids within an internode? Simply two different internodes, or in relation to the bifurcation?
- Why, in general, do polymorphs occur more frequently the longer the 'stem sequence', and ovicells exhibit the converse?
- Why, in *T. inopinata*, are ovicellate autozooids shorter than those which are non-ovicellate?
- Why, in *T. inopinata*, are ovicells rare on sub-apical autozooids?
- In *T. inopinata*, is there a relationship between simple and bifid spines, the extensiveness of scuta, and the presence or absence of ovicells?

- In *S. reptans*, is there a positive association between vibracula and ovicells?
- In *S. reptans*, is there a relationship between the occurrence of frontal avicularia and ovicells? Does that of the former constrain that of the latter?
- In *T. inopinata*, is there a relationship between the occurrence of lateral avicularia and ovicells? Does that of the latter constrain that of the former?
- In *T. inopinata*, why should polymorphs occur more frequently in complete internodes, and ovicells exhibit the converse?

I have speculated, perhaps too much, as to possible benefits accruing from particular arrangements, and there is much scope for further investigation (see below).

7.3.2 Suggestions for further work

There would seem to be two separate areas directly related to this study:-

- Establishing whether or not findings here also applied to other material of the same species, or indeed other similar, and perhaps not so similar species.
- Investigating individual findings, via testable hypotheses, as to why they might be biologically advantageous.

In particular it would be interesting to know to what extent the branching pattern, and colony structure described here applies to other unilaminar biserial cellularines. It may be that the branching pattern occurs much more widely. Are there other substantially different structures, and if so, do they achieve something which this does not? Is *S. scruposa* without any recognisable structure and are there other similar species?

The colonies of the species of this study lent themselves to systematic quantitative investigation by virtue of the fact that the spatial arrangement of their constituent zooids could be recorded as if it occurred in two dimensions. The method used could, and perhaps with careful prior investigation reduced in extent, be applied to other such species.

The extent of variations in the level of occurrence of certain polymorphs in material from three populations of *S. reptans* demands caution in respect of species wide generalisations. At the same time the constant direction of asymmetries of occurrence suggests constancy in a number of respects.

Investigating the numbers and distribution of polymorphic heterozooids and ovicells within a colony is very complex, and is probably best attempted in species where the number of elements (polymorphs or polymorphs and ovicells) is small (as in *T. inopinata*). *S. reptans* is very fascinating in this respect, but very complex.

I said at the outset of this study that that the majority of species descriptions, understandably as they were generally within regional faunal descriptions, were less comprehensive than they might have been. Given the possible occurrence of intra- or inter-colony (or indeed inter-population) variation, caution is obviously necessary before any character is deemed characteristic of a species. It is important that tendencies and trends are identified but are differentiated from absolutely constant characteristics. Nevertheless, this study has highlighted a variety of patterns, trends and correlations in respect of both autozooids (and the larger units in which they are arranged), and polymorphic heterozooids and ovicells, which appear significant if not absolute. Their causality and/or significance are generally unknown, and the extent of their occurrence in other taxa would be invaluable in these respects. A number of these characteristics could be usefully investigated (even if not exhaustively) and referred to in species descriptions. Doubtless others exist, which either I have not detected, or which are not present in the taxa considered here. "Population dynamics of modular organisms should be studied at the level of the intracolony module. This will not only describe the process of development but will also portray the form of modular organisms as a consequence of their internal population dynamics" (Harper and Bell, 1979).

7.4 SUMMARY

In the two species *Scrupocellaria reptans* and *Tricellaria inopinata* this study has revealed previously undescribed fundamental aspects of colonial structure: viz., the arrangement of internodes within 'stem sequences', 'stem sequences' within

'aggregations', and 'aggregations' within a colony. Although colony form was slightly different in the two species the structure was essentially identical in them both. It was thus found in species in two different genera. It was, however, absent, in the limited material I have seen, from a congener (*S. scruposa*) of one of them.

The production in *S. reptans*, of a number of long 'stem sequences' constituted entirely (or virtually so) of a single length of internode, which within the colony overall was in a minority, demonstrates a considerable degree of colonial control of internode length. Further, if the colony can determine when an internode is to bifurcate, it may well, by not triggering a bifurcation, be able to bring an internode and its 'stem sequence' to an end, and thus directly control the extent of growth. In *T. inopinata* long 'stem sequences' consisted of the two shortest length internodes, and the most proximal generations within the colony overall were all of the shortest length. Colonies of both species are essentially, beyond the initial phase of growth, an incomplete circle of incomplete circular structures. The structure and essentially radially symmetrical form of the colonies of both species would seem well suited both to withstand the stresses of water movement, and to maximise the area, and optimise the orientation, of the feeding zooids. The structure results from a combination of a consistent pattern of bifurcations; a pattern of internode and therefore 'stem sequence' termination; and the complex angulation and twisting between internodes at bifurcations.

In relation to the spatial arrangement of polymorphs and ovicells within a colony the results were much less conclusive, and the situation was very different in the two species. For constantly and predictably occurring polymorphs there was no pattern of occurrence to investigate. The study has identified for *S. reptans,* in addition to the axial vibraculum, a second polymorph, the frontal avicularium, whose pattern of occurrence was utterly predictable and therefore genetically determined.

For unpredictably occurring polymorphs the situation was very complex. Much information was assembled in respect of the occurrence, on various scales, of polymorphs and ovicells. Variations in patterns of occurrence are more usefully considered as differential production by autozooids in relation to their position within

an internode, the characteristics of that internode, that of its 'stem sequence', or in relation to the complete colony. The two species are best considered separately although there were characteristics common to both.

In *S. reptans*, for which three populations were investigated, there was evidence of inter-colony and inter-population variation of lateral avicularia, and of inter-population variation of vibracula. In relation to this, patterns which were very clearly defined at low levels of occurrence remained, but less clearly defined, at higher levels. The patterns are significant.

There was a dichotomy between the two series of autozooids within an internode in respect of the polymorphs and ovicells. When most apparent, at low levels of occurrence, the external series contained all of the predictably occurring frontal avicularia, a majority of the lateral avicularia, but very few vibracula and virtually no ovicells. The internal series contained a minority of lateral avicularia, most of the vibracula and virtually all of the sparsely produced ovicells. Whether these asymmetries of occurrence result simply from two different 'handed' internodes, or occur in relation to the bifurcation is unclear. There are observed possible associations, positive and negative, between polymorphs or a polymorph and ovicells, which may be involved. The constant occurrence of a large frontal avicularium on all but the first autozooid in the external series would seem a probable explanation why ovicells, which occupy an identical position on the autozooid, are virtually limited to the internal series. It is possible that vibracula occur predominantly in the internal series because that is where the ovicells are, and that avicularia are concentrated in the external by virtue of their (assumed) defensive function.

In relation to 'stem sequences' both lateral avicularia and vibracula occurred more frequently the longer the 'stem sequence'. Ovicells exhibited the opposite pattern, to a greater extent, and were rare or absent in the long 'stem sequences'.

In *T. inopinata* the situation was simpler with only lateral avicularia and ovicells to consider. Within internodes there was no dichotomy in relation to the two series of

autozooids, but lateral avicularia were concentrated on sub-apical, and ovicells on proximal, autozooids. Lateral avicularia occurred more frequently in complete, stem internodes, and ovicells in incomplete, branch internodes. In relation to 'stem sequences', lateral avicularia occurrence was positively correlated with their length whilst that of ovicells exhibited the converse pattern. Lateral avicularia and ovicells exhibited contrary patterns of occurrence in all respects. This is a counter intuitive situation and it is difficult to imagine in what respect it could be of value. Does it perhaps result from a constraint of some kind? Regarding the relationship between the two, evidence points to ovicells, and therefore embryo producing autozooids, perhaps determining to some degree, the spatial distribution of lateral avicularia. The high level of ovicell occurrence in short 'stem sequences' largely resulted from the simultaneous occurrence of many short 'stem sequences' and the prolific production of ovicells. The higher level of lateral avicularia occurrence in long 'stem sequences' probably resulted from the absence of ovicells. The virtual absence of ovicells in all of the longer 'stem sequences' is a primary feature. Embryo production was therefore largely absent from the most structurally important 'stem sequences', which were generally also constituted of significantly longer autozooids.

There appears to be a very different relationship between polymorphs and ovicells in the two species. In *S. reptans*, ovicell and embryo production is probably constrained by the number and spatial distribution of frontal avicularia. In *T. inopinata* the reverse situation probably obtains with lateral avicularia largely restricted to sites where embryos and ovicells do not occur. There would appear to be considerable colonial control, operating in opposite directions, in the two species. The scenarios accord well with the assumed ecological characteristics of the two species. The most obvious difference between the two species was, as was already known, in the level of their fecundity. This study provides evidence of a dynamic interaction between somatic and reproductive effort in colonial organization.

Consistent patterns of occurrence were apparent, if not always absolute, at all levels within the colony. Those of certain polymorphs persisted through variations in the overall level of occurrence. All such patterns are indicative of order and presumably of colonial organization. They point to the pervasive central role of genetically

determined astogenetic patterns which exhibit varying degrees of plasticity. These must interact with each other and incorporate environmental inputs, to produce the actual arrangement of zooids within a colony. This would seem to need to involve a hierarchical arrangement of 'requirements', which itself may need to be plastic, to adjust to temporal changes within the colony and in the external environment.

7.5 ADDENDUM

A very recent paper, 'Branching and Self-Organisation in Marine Modular Colonial Organisms: a Model' (Sanchez et al., 2004) is of some interest. The authors state, "despite the universality of branching patterns in marine modular colonial organisms, there is neither a clear explanation about the growth of their branching forms nor an understanding of how these organisms conserve their shape during development". The authors continue, "branching in marine modular colonial organisms is both a developmental and ecological process". Using gorgonians the authors endeavour to model not only colony form but also its development. Branching here is less complex than in the bryozoans of this study and branches are described in terms of 'mother' and 'daughter' branches (where the latter develop from the former). The authors argue that colony shape is maintained by the maintenance of a constant ratio between the total number of branches and the number of 'mother' branches.

Their model aimed to "reconcile the intrinsic process of branching, as a 'self-organised criticality', with ecological/physiological size-dependent constraints". They state that, "the [branching] pattern found among these gorgonians is similar to that of systems whose dynamics can be described by a process of self-organised criticality (Bak, 1996)". Such a process consists of dynamic behaviour around a parameter due to the critical effect of that value in the system. The concept is new to me, but may well be involved in some of the probably intrinsically controlled characteristics observed in this study, notably the arrangement of internodes in 'stem sequences', and their arrangement within a colony.

GLOSSARY

Adhesive sac: everted by larva to attach to substrata.

Adventitious avicularium: one developing on the external body wall of an autozooid (cf. vicarious).

Ancestrula: the first zooid(s), of a colony, formed as a result of the metamorphosis of the originally planktonic larva.

Aperture: in ascophorines an opening in the frontal wall not necessarily coextensive with the orifice.

Astogenetic change: the progressive change in zooid morphology related to autozooid generation relative to the ancestrula; generally limited to early generations.

Astogenetic level: the generation of an autozooid relative to the ancestrula.

Astogeny: the development of the colony by budding.

Autozooid: a zooid capable of feeding (cf. heterozooid).

Avicularium (pl. **Avicularia**): a specialised zooid, in the Cheilostomatida, usually with a vestigial polypide and enhanced musculature operating a mandible, a structurally reinforced homologue of the operculum.

Basal: the underside of an encrusting colony, the non-orifice bearing face of all unilaminar colonies (cf. frontal).

Brown body: spherical non-histolysable remains of degenerated polypide.

Capitulum: generally spherical group of autozooids supported on a peduncle of kenozooids.

Coelomopore: a pore connecting the coelom with the exterior.

Cyphonautes: planktotrophic bivalved larva of some bryozoans.

Cystid: cellular and skeletal layers of external body wall of a zooid.

Distal: the side away from the ancestrula (cf. proximal).

Epistome: a projecting flap covering the mouth in Phylactolaemates

Exterior wall: the outer body wall of an autozooid, including the cuticle

Frontal: the upper side of an encrusting colony, the orifice bearing face of all unilaminar colonies (cf. basal).

Frontal membrane: the uncalcified part of the frontal wall in Cheilostomatida.

Funiculus: mesenchymatous strands connecting the polypide with the zooid wall, and then to adjacent zooids.

Gonozooid: modified zooid forming a brood chamber

Gymnocyst: calcified frontal shield of exterior calcification.

Heterozooid: specialised zooid incapable of feeding (cf. autozooid).

Hyperstomial ovicell: rests on or partially embedded in the distal zooid, and opens above the operculum of the parent zooid.

Interior wall: calcified internal body wall of an autozooid, not bounded by cuticle.

Internode: the assembly of zooids between nodes in jointed arborescent species.

Intertentacular organ: tubular extension of the coelomopore by which ova are released in oviparous species.

Kenozooid: heterozooid without a polypide, and usually without an orifice or muscles.

Lophophore: a ring of ciliated tentacles surrounding the mouth of a zooid.

Mandible: reinforced homologue of the operculum in avicularia, powered by enhanced musculature.

Marginal spines: spines often found around the frontal membrane in Cheilostomatida.

Maternal zooid: the zooid producing the ova, (but not necessarily the ovicell).

Nanozooid: dwarf zooid in the Cyclostomata with a reduced polypide with one tentacle.

Operculum: a generally uncalcified lamina which closes the orifice in the vast majority of Cheilostomatida.

Opesia: in the Neocheilostomatina the opening below the frontal membrane which remains after the development of a cryptocyst.

Orifice: the opening in the frontal wall through which the lophophore is everted.

Ovicell: the spherical brood chamber in many Cheilostomatida.

Polypide: the organs and tissues of an autozooid which undergo periodic degeneration and regeneration, i.e. the tentacles, tentacle sheath, alimentary canal, associated musculature and nerve ganglion.

Pore-plate: differentiated zone of a vertical wall pierced by several communication pores, connecting adjacent zooids.

Proximal: refers to the side towards the ancestrula (cf. distal).

Rhizoids: tubular kenozooids which attach upright forms to the substratum, and sometimes interconnect branches of such a colony.

Scutum (pl. **Scuta**)**:** a specialised spine which overarches the frontal membrane in many species of the Candidae.

Septum (pl. **Septa**)**:** an interior wall without cuticle.

Seta (pl. **Setae**)**:** whip-like homologue of the operculum in vibracularia, powered by enhanced musculature.

Spinozooid: kenozooidal spine

Stolon: tubular kenozooids, most commonly in Ctenostomatida, linking autozooids.

Tentacle sheath: part of body wall introverted in lophophores retraction and which then encloses them.

Vibraculum (pl. **Vibracula**)**:** a specialised zooid, in the Cheilostomatida, without a polypide and with enhanced musculature operating a long seta, a homologue of the operculum.

Vicarious avicularium: replaces an autozooid in a series.

Zooecium (pl. **Zooecia**)**:** zooid skeleton.

Zooid: a single bryozoan individual or module.

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APPENDIX 'E'. The lengths, and internode composition and sequence, of 'stem sequences' originating in the first 13 generations of internodes in one half of a colony of *Scrupocellaria reptans* (incomplete internodes denoted *)

Internode generation										
28		1*								
27		5								
26		5								
25		5	2*							
24		5	5							
23		7	5							
22		5	5							
21		5	5							
20		5	5							
19	7	5	5							
18	5	5	5							
17	5	5	5							
16	5	5	5							
15	5	5	5							
14	5	5	5					4*		
13	5	5	5					5		
12	5	5	5			7*		5		
11	5	5	5	5		7		5		
10	5	5	5	5		5		5		
9	5	5	5	5		5		5		
8	5	5	5	5		5	9*	5	6*	
7	5	5	5	5	4*	5	5	5	7	5
6	5	5	5	5	7	5	5	7	7	7
5	5	5	5	5	5	5	5	5		
4	5	5	5	5						
3	5	5								
2	3									
1										
Internode of origin	B 2	C 4	D 5	D 8	E 9	E 12	E 13	E 16	F 17	F 20

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14										
13										
12										
11										
10				8*		3*				
9	7*	1*		7	2*	5		2*		4*
8	5	7	1*	5	7	7		9		7
7	7	7	7	7	7	7	10*	7	5*	5
6	5	5	7	5	7	7				
5										
4										
3										
2										
1										
Generation of origin	F 21	F 24	F 25	F 28	F 29	F 32	G 33	G 37	G 40	G 41

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14										
13										
12										
11							2*		3*	
10	2*						9		7	
9	7					10*	7	7	7	
8	5	7*	7	9	9*	5	7	7	5	
7	5	7	5	7	7	7	7	5	7	10*
6										
5										
4										
3										
2										
1										
Internode of origin	G 44	G 45	G 48	G 49	G 52	G 53	G 56	G 57	G 60	G 61

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14										
13										
12			4*							7*
11			7							5
10			5				7			7
9		7	11			5*	7			7
8	5	9	9	3*	7	5	5	5	2*	7
7										
6										
5										
4										
3										
2										
1										
Internode of origin	H 68	H 73	H 76	H 77	H 81	H 84	H 85	H 88	H 89	H 92

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14										
13										
12										
11										
10										4*
9						2*	11		10*	9
8	6*	5*	6*	7	7*	5	7	9*	7	7
7										
6										
5										
4										
3										
2										
1										
Internode of origin	H 93	H 96	H 97	H 101	H 104	H 105	H 108	H 109	H 112	H 113

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14							7*			
13							7			
12							7			6*
11	2*						7	4*		7
10	7	1*	3*				7	7		7
9	7	9	9		7	3*	7	9	2*	5
8	7	5	7	6*						
7										
6										
5										
4										
3										
2										
1										
Internode of origin	H 117	H 120	H 124	H 125	I 145	I 148	I 149	I 152	I 165	I 169

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14							8*			
13	7						7			
12	5				1*		5			1*
11	5				5		7			7
10	5		2*	4*	5		5			7
9	5	9*	7	5	5	4*	5	4*	4*	7
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	I 172	I 173	I 181	I 184	I 197	I 212	I 213	I 216	I 220	I 221

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14										
13										
12					8*					
11		2*		6*	7			1*		
10		9		7	7	2*	5*	7	9	
9	7	5	4*	7	7	7	5	5	5	4*
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	I 224	I 225	I 228	I 233	I 236	I 237	I 240	I 245	I 248	I 252

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16			5							
15			5							7
14			5							5
13			5						6*	7
12			5						7	7
11			7	8*	4*			5	7	7
10	3*	4*	5	5	7	12*	8*	5	5	5
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	J 289	J 297	J 300	J 301	J 304	J 337	J 340	J 341	J 344	J 364

Internode generation										
28										
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24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14										
13										
12										2*
11	3*	5					2*			9
10	5	7	5	7	3*	6*	7	5*	5	5
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	J 393	J 428	J 437	J 441	J 444	J 449	J 465	J 468	J 469	J 472

Internode generation										
28										
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24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14										
13										
12										5*
11	6*		4*	7				2*	7	7
10	5	4*	7	7	7*	5*	6*	9	5	
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	J 476	J 480	J 489	J 492	J 496	J 505	J 508	J 509	J 512	K 596

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19		4*								
18	4*	5								
17	7	7								
16	5	7								
15	7	5								
14	7	7								
13	5	7						7		
12	7	7				1*		9		
11	7	7	3*	7*	1*	7	3*	5	5	13*
10										
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	K 597	K 600	K 601	K 604	K 608	K 676	K 677	K 681	K 684	K 685

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14										
13										
12	7	5								
11	7	7	1*	7*	3*	4*	1*	2*	2*	1*
10										
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	K 688	K 728	K 729	K 732	K 785	K 788	K 900	K 929	K 932	K 933

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14	2*					7*				
13	7		9			7	6*			
12	7		7			5	7			4*
11	5	6*	7	4*	1*	5	5	9	5	7
10										
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	K 940	K 941	K 944	K 952	K 980	K 981	K 984	K 1013	K 1021	K 1024

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16			4*			4*				
15			7	7*		7				
14			7	7	4*	7				3*
13	7*		7	7	7	7			8*	13
12	7	8*	7	7	7	7	4*	3*	9	7
11										
10										
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	L 1189	L 1192	L 1193	L 1196	L 1197	L 1200	L 1205	L 1349	L 1364	L 1368

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14									2*	
13			2*		7*				9	5*
12	10*	5*	7	7*	7	4*	9*	4*	7	7
11										
10										
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	L 1373	L 1376	L 1452	L 1453	L 1456	L 1461	L 1701	L 1704	L 1873	L 1876

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14				2*						
13	10*		6*	7	9		6*	1*		
12	7	6*	5	7	5	7*	7	5	6*	3*
11										
10										
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	L 1880	L 1884	L 1888	L 1961	L 1964	L 1965	L 1968	L 2041	L 2044	L 2045

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17				3*						
16				7		7*				
15		4*		7		7				
14		7	3*	7		5	7	5*	4*	4*
13		7	7	5	5	7	7	5	9	7
12	4*									
11										
10										
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	L 2048	M 2377	M 2380	M 2385	M 2388	M 2389	M 2392	M 2393	M 2396	M 2397

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16				4*	6*					
15	7*		4*	5	7			7*		7*
14	7		9	7	7	1*		9		7
13	7	7	7	5	11	9	7	7	5*	7
12										
11										
10										
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	M 2400	M 2724	M 2725	M 2732	M 2733	M 2736	M 2741	M 2744	M 2748	M 2908

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16				5*						
15				9			3*			
14			6*	7		7	9			7
13	4*	6*	7	5	5	7	7	7*	9*	7
12										
11										
10										
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	M 2912	M 3404	M 3412	M 3413	M 3416	M 3417	M 3420	M 3421	M 3424	M 3745

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14	7*	3*			4*		6*	2*		
13	9	7	7	8*	5	3*	7	7	3*	8*
12										
11										
10										
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	M 3749	M 3752	M 3757	M 3760	M 3773	M 3776	M 3921	M 3924	M 3925	M 3928

Internode generation										
28										
27										
26										
25										
24										
23										
22										
21										
20										
19										
18										
17										
16										
15										
14										
13	6*	6*								
12										
11										
10										
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	M 3932	M 3933								

APPENDIX 'I' Ovicell occurrence related to autozooid number; based on 300 internodes, each of which contained at least one ovicellate autozooid, selected at random from a colony of *Scrupocellaria reptans* collected at Swanage, 130701

P = present; X= not possible here; I= incompletely developed (end of internode).

Autozooid number														
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	P		P			X								
	P					X								
	P				P					X				
	P		P*											
	P			I	I									
	P	I	I											
	P		P	I	I									
	P			I	I									
			P			X								
			P			X								
	P		P	I	I									
	P					X								
	P			X										
	P		P		P	X								
	P			X										
	P					X								
			P		P	X								
			P			X								
	P		P		I	I								
	P		P	X										
	P			I	I									
	P		P			X								
			P		I	I								
	P		I	I										
	P		P			X								
	P		P		P	I	I							
	P					X								
	P		P	I	I									
	P		I	I										
	P					X								
	P					X								
	P		P	X										
			P			X								
	P				P					I	I			
	P		P	X										
	P					X								

	P		P	X										
	P		P		P	X								
	P	I	I											
	P			X										
	P		I	I										
	P					X								
	P	I	I											
	P		P		P	X								
	P		P		P	I	I							
	P		P			X								
	P		P	I	I									
			P		P			X						
	P			X										
	P		P			X								
	P		P			X								
	P		P				I	I						
	P				I	I								
			P		I	I								
	P		P			X								
	P				I	X								
			P		P	X								
	P		I	I										
	P		P					X						
	P			X										
	P					X								
	P		P			X								
	P		P			X								
			P		P	X								
	P		P			X								
	P			X										
	P		P			X								
	P					X								
	P		P			X								
	P		P			X								
	P		P			X								
	P					I	I							
	P			X										
	P		I	I										
			P			I	I							
			P	I	I									
	P		P	X										
	P		P			X								
	P			X										
	P		P				P		P			X		

	P		P	X										
	P		P	X										
	P		P		I	I								
	P		P			X								
	P		P	X										
	P			X										
			P			X								
	P		P	X										
	P			X										
	P			X										
	P		P		P	X								
	P		P	X										
			P			X								
	P		P			X								
					P		P	I						
	P			I	I									
	P		P		I	I								
	P		P			X								
	P		P	X										
	P	I	I											
	P		P		P			X						
			P					X						
	P		P			I	I							
	P		P		P		P			I	I			
	P		P		P	X								
	P		P		P	X								
			P					X						
			P			I	I							
			P											
			P											
	P		P	X										
	P		P			X								
	P		P		P	X								
	P		I	I										
	P		P			X								
	P		P		I	I								
			P			X								
	P		P		P	X								
	P		P		P	I	I							
	P		P	I										
	P				P	I	I							
	P		P		I	I								
	P			X										
	P		P	I	I									
	P		P		P		I	I						
	P		P			X								

	P	I	I											
	P		P		P	I	I							
			P		P		I	I						
	P		P	X										
	P		P		P			X						
	P		I	I										
	P		P			X								
			P		P	X								
	P			I	I									
					P	X								
	P		P		P		I	I						
	P		P		I	I								
	P		P		P			X						
			P					X						
			P		P		I	I						
	P		P	X										
	P				P	I								
	P		P		P				I	I				
	P			X										
	P		P		P	X								
	P		P		P	X								
	P		P			X								
	P		P			I	I							
			P			X								
	P		P		P	I	I							
			P			X								
	P					X								
	P		P			I	I							
	P			I	I									
	P			X										
	P		P			X								
	P		P		P	X								
	P		P		P			X						
	P			I	I									
	P		P	X										
	P		P		P	X								
	P		P			I	I							
	P			I	I									
	P		P			X								
	P			X										
	P		P		P	X								
	P		P		P			X						
	P		P	I	I									
	P		P			X								
	P		P		P	X								
	P		P		P			X						

	P		P					X						
	P		P		P			X						
	P			I	I									
	P		P	I	I									
	P		P		I	I								
	P		P	X										
	P			I	I									
	P					X								
	P		P	I	I									
	P		P	I	I									
	P		P	X										
	P		P		P		I	I						
	P			X										
	P		P					X						
	P		P			I	I							
	P		P	X										
	P		P		P	X								
	P		P			I	I							
	P			I	I									
	P		P			X								
	P		P			X								
	P		P			X								
	P		P			X								
	P		I	I										
	P		I	I										
	P		P			X								
	P		P			X								
			P			X								
	P			X										
	P		P		P	X								
			P			X								
	P				I	I								
	P		P		P	X								
	P			X										
	P		P		P			X						
					P	X								
	P			X										
	P			X										
	P		P	X										
	P		P	X										
	P			X										
	P		P*											
	P	I	I											
	P			X										
	P		P			X								
	P		P			X								

	P			X										
	P		P	I	I									
	P		P			I	I							
	P		P		P		P	X						
	P		P			X								
	P					X								
	P			I	I									
	P		P	I	I									
			P			X								
	P		P			I	I							
	P		P			X								
					P			X						
			P	I	I									
	P		P		P	X								
			P			I	I							
			P			X								
	P		P		P	X								
	P		P			X								
	P		P			X								
	P									X				
	P			X										
	P		P	X										
	P					X								
	P		P			X								
	P	I	I											
					P			X						
	P		P		P		P		P					X
			P				P					X		
	P		P		P	I	I							
	P		P			X								
	P			X										
	P		P			X								
	P		P			X								
	P		P			X								
			P				P		P			X		
	P		P	X										
	P		P		P			X						
	P		P		P			I	I					
	P			X										
	P					X								
	P		I	I										
			P			X								
	P		P			X								
	P		P			X								
	P		P		P		P	X						
			P				P	P	P		P			X

	P		P		P	P	P		P	X				
	P	P	P		P	X								
		P	P			X								
	P		P			X								
	P	P	P		P	X								
	P	P	P		P	I	I							
	P		P	P	P				P	P				X
	P	P	P	I	I									
	P	P	P	P	P		P	X						
	P	P	P		P		I	I						
		P	P	P			P		P					X
	P	P	P		P	X								
	P	P	P		P	X								
	P	P	P		P	X								
	P						P			X				
	P		I	I										
	P			I	I									
	P		P		P			X						
	P						P			X				
	P			I	I									
	P		P					I	I					
	P				I	I								
	P			X										
	P		P			X								
			P		P			X						
	P		P			X								
	P		P			X								
	P		P		P			X						
	P		P			X								
	P		P		P	X								
	P		P		P	X								
	P			X										
	P		P			X								
			P			X								

APPENDIX 'K' The lengths, and internode composition and sequence, of 'stem sequences' originating in the first10 generations of internodes in a colony of *Tricellaria inopinata* (incomplete internodes *)

Internode generation										
15	5*									
14	5									
13	5	5		5		2*				
12	3	5		5		5				
11	5	3		3		5	2*	7		
10	3	5		5	7	3	5	5		5
9	5	3		3	5	5	5	3		5
8	3	5	3*	5	5	5	5	5		5
7	5	3	3	3	5	3	3	3		5
6	3	3	3	5	5	5	5	5		5
5	3	3	3	3	3	3	3	3	3	3
4	3	3	3	3	3	3	3	3		
3	3	3	3	3						
2	3	3								
1	3									
Internode of origin	A 1	B 2	C 1	C 4	D 1	D 4	D 5	D 8	E 1	E 4

Internode generation										
15										
14										
13										
12										
11									5*	
10				6*					5	
9				3					5	
8				5					5	6*
7				3		11*		2*	5	5
6	5			5		7	8*	11	3	3
5	3	3	1*	3	3	3				
4										
3										
2										
1										
Internode of origin	E 5	E 8	E 9	E 12	E 13	E 16	F 1	F 4	F 5	F 9

Internode generation										
15										
14										
13										
12										
11				1*						
10	4*		1*	5						
9	3		7	5						
8	5	6*	5	3	11*	9*		9*	8*	
7	3	7	5	5	7	3	5*	5	7	2*
6	3	3	3	3	3	5	3	3		
5										
4										
3										
2										
1										
Internode of origin	F 12	F 13	F 20	F 21	F 24	F 25	F 28	F 29	G 5	G 8

Internode generation										
15										
14						5*				
13						5				
12						5				
11						5		4*		
10					2*	5		5		
9		8*			7	3		5		
8	10*	7			7	5		5		
7	7	5	9	8*	3	3	6*	3	7	6*
6										
5										
4										
3										
2										
1										
Internode of origin	G 9	G 12	G 13	G 17	G 20	G 21	G 25	G 28	G 37	G 40

Internode generation										
15										
14										
13										
12		5								
11		5				5				
10	4*	5				5				
9	7	3	4*			5			2*	
8	5	5	11		9*	5			5	
7	3	3	3	11*	5	3	14*	14*	3	7*
6										
5										
4										
3										
2										
1										
Internode of origin	G 41	G 44	G 45	G 48	G 49	G 53	G 56	G 57	G 60	G 61

Internode generation										
15										
14										
13										
12					5*					
11					5					
10					5					
9		5			5					
8	9	7	4*	12*	3	2*	7*	4*	2*	4*
7										
6										
5										
4										
3										
2										
1										
Internode of origin	H 9	H 12	H 17	H 20	H 21	H 24	H 25	H 28	H 36	H 40

Internode generation										
15										
14										
13										
12	5*	2*								
11	5	5						4*		
10	5	3	9					5		
9	5	5	5		5*			5		
8	3	3	3	6*	3	3*	5*	3	7*	15*
7										
6										
5										
4										
3										
2										
1										
Internode of origin	H 41	H 44	H 45	H 52	H 53	H 56	H 73	H 76	H 77	H 81

Internode generation										
15										
14										
13										
12										
11		9								5
10		3	9*		6*					5
9	9*	5	7		7				6*	5
8	3	3	3	12*	3	8*	6*	10*	11	3
7										
6										
5										
4										
3										
2										
1										
Internode of origin	H 84	H 85	H 88	H 89	H 92	H 93	H 97	H 100	H 105	H 108

Internode generation										
15										
14										
13										
12										
11		5*								
10		5					8*			
9		5		10*	5*	10*	7	6*	7*	11*
8	8*	3	5*							
7										
6										
5										
4										
3										
2										
1										
Internode of origin	H 116	H 117	H 120	I 21	I 24	I 37	I 41	I 45	I 53	I 77

Internode generation										
15										
14										
13										
12							5			
11			2*		2*	4*	5			
10		13*	5		5	5	5		5*	
9	4*	5	3	4*	3	5	3	6*	5	4*
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	I 81	I 84	I 85	I 89	I 92	I 105	I 108	I 109	I 149	I 152

Internode generation										
15										
14										
13										
12						5				
11					9	3				
10			10*		7	5				6*
9	7*	8*	7	9*	3	3	11*	12*	4*	3
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	I 156	I 164	I 165	I 168	I 169	I 172	I 173	I 176	I 179	I 182

Internode generation										
15										
14										
13										
12										
11			1*							
10	6*		5	3*		5		5*	7	9
9	7	8*	3	7	6*	3	7*			
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	I 183	I 212	I 213	I 216	I 233	I 236	I 237	J 44	J 76	J 81

Internode generation										
15							4*			
14							5			
13							5			
12				2*			5			
11				9			5	8*	1*	
10	11	1*	4*	7	2*	3*	3	5	9	1*
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	J 84	J 156	J 164	J 165	J 168	J 169	J 172	J 173	J 180	J 181

Internode generation										
15										
14										
13										
12										
11				5*			4*			
10	4*	8*	1*	7	2*	5*	3	6*	3*	3*
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	J 184	J 209	J 213	J 216	J 220	J 297	J 300	J 301	J 325	J 329

Internode generation										
15										
14										
13				9					7*	
12				5		3*			5	
11			8*	5		5		7*	5	5*
10	2*	2*	5	3	7*	5	4*	5	3	5
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	J 332	J 337	J 340	J 341	J 361	J 364	J 365	J 425	J 428	J 429

Internode generation										
15										
14										
13										
12										
11										
10	5*	8*	3	9						
9										
8										
7										
6										
5										
4										
3										
2										
1										
Internode of origin	J 432	J 468	J 469	J 472						