



Swansea University
Prifysgol Abertawe



Cronfa - Swansea University Open Access Repository

This is an author produced version of a paper published in:

Molecular Ecology

Cronfa URL for this paper:

<http://cronfa.swan.ac.uk/Record/cronfa44683>

Paper:

Wells, D., Cant, M., Nichols, H. & Hoffman, J. (2018). A high-quality pedigree and genetic markers both reveal inbreeding depression for quality but not survival in a cooperative mammal. *Molecular Ecology*, 27(9), 2271-2288. <http://dx.doi.org/10.1111/mec.14570>

This item is brought to you by Swansea University. Any person downloading material is agreeing to abide by the terms of the repository licence. Copies of full text items may be used or reproduced in any format or medium, without prior permission for personal research or study, educational or non-commercial purposes only. The copyright for any work remains with the original author unless otherwise specified. The full-text must not be sold in any format or medium without the formal permission of the copyright holder.

Permission for multiple reproductions should be obtained from the original author.

Authors are personally responsible for adhering to copyright and publisher restrictions when uploading content to the repository.

<http://www.swansea.ac.uk/library/researchsupport/ris-support/>

MOLECULAR ECOLOGY

A high quality pedigree and genetic markers both reveal inbreeding depression for quality but not survival in a cooperative mammal

Journal:	<i>Molecular Ecology</i>
Manuscript ID	MEC-18-0035.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	05-Mar-2018
Complete List of Authors:	Wells, David; Universitat Bielefeld Fakultat fur Biologie, animal behaviour; Liverpool John Moores University, Natural sciences and psychology Cant, Michael ; University of Exeter, College of Life and Environmental Sciences Nichols, Hazel; Liverpool John Moores University, School of Natural Science and Psychology Hoffman, Joe; University of Bielefeld, Animal Behaviour
Keywords:	Inbreeding, Heterozygosity, Reproductive success, Survival, Pedigree, Banded mongoose

1 **A high quality pedigree and genetic markers both reveal inbreeding depression for**
2 **quality but not survival in a cooperative mammal**

3

4 David A. Wells^{1,2}, Mike A. Cant³, Hazel J. Nichols^{2*}, Joseph I. Hoffman^{1*}

5

6 1. Department of Animal Behaviour, University of Bielefeld, Postfach 100131, 33501

7 Bielefeld, Germany

8 2. School of Natural Science and Psychology, Liverpool John Moores University, Liverpool

9 L3 3AF, UK

10 3. College of Life and Environmental Sciences, University of Exeter, Penryn TR10 9FE, UK

11 * Joint senior authors

12

13 *Keywords:* Inbreeding depression, heterozygosity, reproductive success, survival,

14 microsatellites, cooperative breeding, banded mongoose

15

16 Corresponding author: David A. Wells

17 Email: david.wells@uni-bielefeld.de, d.a.wells@2016.ljmu.ac.uk

18

19 Running title: Inbreeding depression in banded mongooses

20

21 **Abstract**

22

23 Inbreeding depression, the reduced fitness of offspring of closely related parents, is
24 commonplace in both captive and wild populations and has important consequences for
25 conservation and mating system evolution. However, because of the difficulty of collecting
26 pedigree and life history data from wild populations, relatively few studies have been able to
27 compare inbreeding depression for traits at different points in the life cycle. Moreover,
28 pedigrees give the expected proportion of the genome that is identical by descent (IBD_g)
29 whereas in theory with enough molecular markers realised IBD_g can be quantified
30 directly. We therefore investigated inbreeding depression for multiple life-history traits in a
31 wild population of banded mongooses using pedigree-based inbreeding coefficients (f_{ped}) and
32 standardised multilocus heterozygosity (sMLH) measured at 35–43 microsatellites. Within an
33 information theoretic framework, we evaluated support for either f_{ped} or sMLH as inbreeding
34 terms and used sequential regression to determine whether the residuals of sMLH
35 on f_{ped} explain fitness variation above and beyond f_{ped} . We found no evidence of inbreeding
36 depression for survival, either before or after nutritional independence. By contrast,
37 inbreeding was negatively associated with two quality related traits, yearling body mass and
38 annual male reproductive success. Yearling body mass was associated with f_{ped} but not
39 sMLH, while male annual reproductive success was best explained by both f_{ped} and residual
40 sMLH. Thus, our study not only uncovers variation in the extent to which different traits
41 show inbreeding depression, but also reveals trait-specific differences in the ability of
42 pedigrees and molecular markers to explain fitness variation and suggests that for certain traits
43 genetic markers may capture variation in realised IBD_g above and beyond the pedigree
44 expectation.

45

46 **Introduction**

47

48 Inbreeding depression, the reduction in offspring fitness that can result from incestuous
49 matings, occurs in a wide range of both captive and wild populations (Hedrick & Garcia-
50 Dorado, 2016; Keller & Waller, 2002). Inbreeding increases the proportion of the genome
51 that is identical by descent (IBD_g), which in turn reduces fitness mainly through the increased
52 expression of deleterious recessive alleles but also due to increased homozygosity at loci
53 showing overdominance (Charlesworth & Willis, 2009). The resulting loss in fitness can be
54 substantial and is believed to have shaped the evolution of dispersal and mating behaviour in
55 many species. Consequently, quantifying the severity of inbreeding depression in natural
56 populations is essential for understanding population and evolutionary dynamics (Hedrick &
57 Garcia-Dorado, 2016; Keller & Waller, 2002; Nichols, 2017; Szulkin, Stopher, Pemberton, &
58 Reid, 2013).

59

60 Inbreeding depression is predicted to be strongest for traits that are closely related to fitness
61 such as survival and reproduction, as these will be subject to stronger directional selection and
62 therefore exhibit greater directional dominance (Falconer & Mackay, 1996). This is
63 supported by a meta-analysis of 54 animal species, although most of the studies involved were
64 of captive or experimental populations (DeRose & Roff, 1999). However, understanding how
65 inbreeding depression affects different life history traits in natural populations is more
66 challenging due to the difficulty of collecting high quality lifetime fitness measures and
67 generating deep, well resolved pedigrees. Furthermore, strong viability selection against
68 inbred offspring will result in an adult population in which inbred individuals are rare,

69 potentially making it more difficult to detect inbreeding depression for late acting traits
70 (Huisman, Kruuk, Ellis, Clutton-Brock, & Pemberton, 2016).

71

72 Traditionally, pedigrees were considered the gold standard for measuring inbreeding in
73 natural populations (Pemberton, 2004). However, the vast majority of pedigrees are
74 incomplete and will also contain errors that can impair their ability to detect inbreeding
75 depression (Reid et al., 2014; H. R. Taylor, Kardos, Ramstad, & Allendorf, 2015).

76 Additionally, pedigrees cannot account for inbreeding caused by ancestors who are not
77 included in the pedigree. This can result in downwardly biased estimates of inbreeding,
78 particularly where the pedigree is only a few generations deep and relationships among the
79 founders are unknown (Kardos, Luikart, & Allendorf, 2015). Arguably, an even greater issue
80 is that pedigrees simply cannot be generated for the majority of wild populations, many of
81 which are large and demographically open.

82

83 A further drawback of pedigrees is that, even when multiple generations of accurate ancestry
84 data can be collected, the pedigree inbreeding coefficient (f_{ped}) quantifies an individual's
85 *expected* IBD_g based on the known common ancestors of its parents, whereas *realised* IBD_g
86 will differ stochastically from this expectation due to Mendelian segregation and
87 recombination (Hedrick, Kardos, Peterson, & Vucetich, 2016; Hill & Weir, 2011; Knief,
88 Kempenaers, & Forstmeier, 2016). The variance in realised IBD_g among individuals with the
89 same f_{ped} will be higher for species with few chromosomes and short genetic maps (Fisher,
90 1965; Franklin, 1977; Hill & Weir, 2011; Kardos et al., 2015) and will also decrease with the
91 number of generations separating an inbred individual from its common parental ancestor(s)

92 as IBD chromosomal segments are gradually broken down by successive recombination
93 events (Hedrick et al., 2016).

94

95 As deep, high quality pedigrees are also lacking for the majority of natural populations, many
96 studies have used the heterozygosity of small panels of typically around 10–20 presumed
97 neutral markers such as microsatellites as a surrogate measure of IBD_g . The result is a large
98 and expanding literature describing heterozygosity-fitness correlations (HFCs) covering a
99 long list of traits and species (Chapman, Nakagawa, Coltman, Slate, & Sheldon, 2009).

100 However, estimates of IBD_g based on such small panels of markers will tend to have limited
101 precision due to both high sampling variance and the difficulty of distinguishing identity by
102 descent (IBD) from identity by state (IBS, Balloux, Amos, & Coulson, 2004; Slate et al.,
103 2004). Recent simulation and empirical studies suggest that these issues can be overcome
104 with very large panels of markers, with around ten thousand or more single nucleotide
105 polymorphisms (SNPs) being preferable under most circumstances even to a deep pedigree
106 for quantifying inbreeding depression (Hoffman et al., 2014; Huisman et al., 2016; Kardos et
107 al., 2015; Wang, 2016). However, until SNP genotyping costs fall to the point where such
108 large datasets can be collected within the budgets of most projects, it is likely that
109 microsatellites will continue to be used to investigate inbreeding effects in wild populations.

110

111 Only a handful of studies have directly compared the ability of f_{ped} and microsatellites to
112 detect inbreeding depression (e.g. Grueber, Waters, & Jamieson, 2011; S. S. Taylor et al.,
113 2010), and these have uncovered mixed results. At one end of the spectrum, Nietlisbach et al.
114 (2017) used an unusually deep and well resolved song sparrow pedigree to show that f_{ped}
115 outperformed microsatellite heterozygosity, even when the latter could be calculated from an

116 unusually large panel of 160 markers. At the other end, both Forstmeier et al. (2012) and
117 Hammerly et al. (2013) found that smaller panels of around ten microsatellites explained more
118 fitness variation than f_{ped} . These contradictory outcomes probably reflect a multitude of
119 factors including variation among studies in pedigree depth and quality, marker number and
120 resolution, as well as factors intrinsic to a given system such as the recombination landscape.
121 Consequently, in order to obtain a more general picture of how pedigrees and genetic markers
122 can capture fitness variation, similar studies of a wider variety of taxa are needed.

123

124 A related question is whether the heterozygosity of genetic markers can explain fitness
125 variation above and beyond that explained by f_{ped} . Some studies have approached this
126 question by testing for HFCs within individuals of the same pedigree inbreeding class
127 (Hansson, Westerdahl, Hasselquist, Åkesson, & Bensch, 2004; Hemmings, Slate, & Birkhead,
128 2012), while others have constructed statistical models of the focal traits containing both f_{ped}
129 and marker heterozygosity (e.g. Bensch et al., 2006), an approach that Nietlisbach et al.
130 (2017) recently termed 'residual heterozygosity-fitness correlation'. However, if these two
131 inbreeding measures are strongly correlated, the variance explained by either term cannot be
132 properly partitioned due to collinearity (Dormann et al., 2013). One way to account for this
133 would be to take the residuals of marker heterozygosity on f_{ped} and fit this as an explanatory
134 variable alongside f_{ped} . The variance shared by these two terms will be attributed to the
135 pedigree, while any effect of residual heterozygosity will reflect the ability of the markers to
136 detect variation in realised IBD_g that cannot be captured by the pedigree. This approach is
137 known as 'sequential regression' (Graham, 2003) or sometimes 'residual regression' and has
138 been shown to perform well in a comparison of approaches for dealing with collinearity
139 (Dormann et al., 2013).

140

141 A long term study of banded mongooses (*Mungos mungo*) provides an excellent opportunity
142 to investigate the strength of inbreeding depression for multiple traits, as well as to explore the
143 ability of f_{ped} and marker heterozygosity to capture fitness variation in a wild vertebrate
144 population. Banded mongooses live in social groups of 10–40 adults and, unlike most
145 cooperative breeders, members of both sexes habitually breed within their natal pack despite
146 the presence of close relatives (Nichols, Cant, Hoffman, & Sanderson, 2014). As a result,
147 inbreeding appears to be common despite evidence that females attempt to avoid inbreeding
148 and that males preferentially mate guard more distant relatives (Sanderson, Wang, Vitikainen,
149 Cant, & Nichols, 2015). Furthermore, inbreeding appears to have fitness implications for
150 offspring as recent studies have uncovered inbreeding depression for both yearling body mass
151 and parasite load (Mitchell, Vitikainen, Wells, Cant, & Nichols, 2017; Sanderson et al., 2015).
152 However, although both of these studies were based on a high quality, nine-generation deep
153 pedigree, only the latter compared the ability of f_{ped} and microsatellite heterozygosity to detect
154 inbreeding depression.

155

156 Here, we genotyped an additional 192 individuals at 35 microsatellite loci in order to enlarge
157 the existing banded mongoose pedigree to include 777 individuals with all four grandparents
158 known. The resulting dataset was then used to investigate inbreeding depression for a variety
159 of traits acting at different time points in the life cycle: (i) survival to nutritional
160 independence; (ii) survival beyond nutritional independence; (iii) yearling body mass; and (iv)
161 annual reproductive success. We additionally evaluated the abilities of f_{ped} , marker
162 heterozygosity and residual marker heterozygosity to detect inbreeding depression. We
163 hypothesised that viability selection against inbred individuals would reduce both the mean

164 and variance in inbreeding in the adult population, thereby rendering inbreeding depression
165 for late-acting traits more difficult to detect. We also hypothesised that, despite having a high
166 quality pedigree, our moderately large panel of microsatellites would allow us to explain
167 fitness variation above and beyond that explained by f_{ped} , and that the explanatory power of
168 the markers would increase with the number of loci.
169

For Review Only

170 **Materials and methods**

171

172 *Study site, individual identification and sample collection*

173 This study was conducted on a free-ranging population of banded mongooses in Queen
174 Elizabeth National Park, Uganda (0°12'S, 27°54'E). The study area comprises approximately
175 10 km² of savannah on and around the Mweya Peninsula and a weather station near the centre
176 measures the amount of daily rainfall. Genetic, behavioural and life-history data were
177 collected from a total of 1,978 individuals between May 1997 and July 2016 inclusive. At
178 any one time, the population consisted of approximately 250 individuals belonging to 10–12
179 social groups. A combination of approaches were used to identify individuals in the field.
180 The majority of individuals were first captured as pups and given either a unique tattoo or a
181 subcutaneous pit tag (TAG-P-122IJ, Wyre Micro Design Ltd., UK) to allow permanent
182 identification. For genetic analysis, a 2mm tissue sample was taken from the tip of the tail
183 using surgical scissors and a dilute solution of potassium permanganate was applied to
184 minimise infection risk. To identify individual mongooses by sight, commercially available
185 hair dye (L'Oreal, UK) was used to apply unique patterns to animals up to six months of age.
186 Adults were given a unique shave pattern and, after they had stopped growing, were fitted
187 with colour-coded plastic collars. To maintain dye markings, shave patterns and collars, all
188 individuals were trapped every 3–6 months as described by Cant (2000), Hodge (2007) and
189 Jordan et al. (2010).

190

191 *Life history data collection*

192 Detailed behavioural and life history data were collected by visiting each pack every 2–4
193 days. All individuals in the population were habituated to human observers. Mongoose packs

194 could be reliably located because one or two adults in each pack were fitted with a 27g radio
195 collar (<2% of body mass, Sirtrack Ltd., New Zealand) with a 20cm whip antenna (Biotrack
196 Ltd., UK). Age could be determined for the majority of individuals born within the study site
197 based on their mother's parturition dates, but was unknown for immigrants. Individual lifespan
198 was calculated as the time in days between the date of birth and the date of death. Death could
199 be distinguished from dispersal because mongooses disperse in groups (Cant, Otali, &
200 Mwanguhya, 2001) and dispersal events are also generally preceded by a period of aggression
201 from the rest of the group (Thompson et al., 2016).

202

203 Escorting is a form of care unique to banded mongooses that affects offspring fitness (Cant,
204 Vitikainen, & Nichols, 2013; Gilchrist, 2004; Hodge, 2005). Escorting begins approximately
205 27 days after birth, when pups leave the den and begin to forage with the pack (Gilchrist,
206 2004). During this time, some of the pups form an exclusive one-to-one relationship with an
207 adult who feeds, grooms, carries and protects them from predators. We therefore collected
208 detailed data on escorting behaviour so that we could incorporate escorting into our analyses
209 of early-acting fitness traits. Throughout the escorting period, which lasts approximately two
210 months, we visited packs once or twice daily. If an adult was closely associated with a pup
211 (i.e. spent more than half of a 20 minute observation period within 0.5m of the focal pup) the
212 adult was deemed to be an escort for that pup. For each pup, we quantified the amount of care
213 received as the proportion of visits during which a pup was seen with an escort.

214

215 *Ethical statement*

216 Research was carried out under licence from the Uganda National Council for Science and
217 Technology and all procedures were approved by the Uganda Wildlife Authority. All research

218 procedures adhered to the ASAB Guidelines for the Treatment of Animals in Behavioural
219 Research and Teaching and were approved by the Ethical Review Committee of the
220 University of Exeter. Our trapping procedure has been used over 8000 times and tissue
221 samples have been taken from over 1900 individuals with no adverse effects.

222

223 *DNA extraction and microsatellite genotyping*

224 Prior to this study, genetic data were available for 1,748 individuals that were tissue sampled
225 between 1997 and 2013 and genotyped at up to 43 microsatellite loci (Sanderson et al. 2015).
226 All of these loci are known to be in Hardy-Weinberg and linkage equilibrium in the study
227 population (Sanderson et al. 2015). To enlarge this dataset, we genotyped an additional 192
228 individuals that were sampled between 2014 and 2015 at 35 of these microsatellites. We
229 excluded 8 loci that had previously been amplified individually and visualised through
230 radioactive incorporation but which failed to amplify reliably in multiplexed PCRs using
231 fluorescent labelled primers. DNA was extracted using Qiagen® DNeasy blood and tissue kits
232 following the manufacturer's protocol. The genotyping was conducted as described in detail
233 by Sanderson et al. (2015). Briefly, fluorescently labelled microsatellite primers were
234 incorporated into seven separate multiplexes. PCR reactions were conducted using a Type It
235 kit (Qiagen) according to the manufacturer's protocol with an annealing temperature of 57°C
236 and a reaction volume of 12µl. PCR products were resolved by electrophoresis on an ABI
237 3730xl capillary sequencer and allele sizes were scored using GeneMarker version 1.95
238 (SoftGenetics, Pennsylvania, USA).

239

240 *Pedigree construction*

241 The resulting microsatellite dataset was used to update an existing banded mongoose
242 pedigree, comprising 1,748 individuals genotyped at 35–43 microsatellite loci (Sanderson et
243 al., 2015). We followed the protocol of Sanderson et al. (2015) to extend the pedigree using a
244 combination of MasterBayes (Hadfield, Richardson, & Burke, 2006) and COLONY (Jones &
245 Wang, 2010). MasterBayes was used as the primary parentage assignment program because
246 of its ability to incorporate phenotypic data, which can result in larger numbers of higher
247 confidence assignments. COLONY was used both to confirm the MasterBayes assignments
248 and to assign sibships among individuals with one or both unsampled parents. The latter
249 provides putative information about the relationships among founders and immigrants rather
250 than assuming that they are unrelated.

251

252 For the MasterBayes analysis, we specified the following strict requirements for assigning
253 parentage: (i) fathers had to be alive on the estimated date of conception of the focal pup; (ii)
254 mothers had to be alive on the date of birth and present in the pack where the focal pup was
255 born; (iii) both parents had to be at least six months of age during the month of conception of
256 the focal pup; (iv) offspring could not be their own parents. To maximise confidence in
257 parentage assignments, we also incorporated the following phenotypic data: (i) age and age²,
258 as reproduction increases with age before tailing off later in life (Sanderson et al., 2015); (ii)
259 whether a female was recorded as having given birth within four weeks of the month in which
260 the pup was born; (iii) whether the male was present in the offspring's pack during the month
261 of conception. MasterBayes was run for 9,772,000 iterations with a burn in of 750,000 and a
262 thinning interval of 9,022. In order to keep the Metropolis Hastings acceptance rate between
263 0.2 and 0.5, the tuning parameters were set to tunePed (beta=0.3, USdam= 0.03,
264 USsire=0.03). Successive samples from the posterior distribution had low autocorrelation ($r <$

265 0.1). MasterBayes parentage assignments were accepted if they had an associated probability
266 greater than or equal to 0.8, although the average assignment probability was 0.99.

267

268 Additionally, COLONY was used to assign individuals to full- and half-sibship groups.
269 Candidate parent and exclusion parent lists for input into COLONY were generated using the
270 same criteria as for MasterBayes. No maternal or paternal sibships were excluded. We
271 specified a sibship prior of 1.5 for both maternal and paternal average sibship size. This was
272 based on prior knowledge of the breeding system and helped to prevent COLONY from
273 incorrectly grouping offspring into large clusters of false siblings. The probability of a true
274 parent being in the candidate list was set to 0.8 and COLONY assignments were only
275 accepted if they had a probability greater than or equal to 0.8. MasterBayes parentage
276 assignments were accepted first and COLONY assignments were then added where
277 MasterBayes failed to confidently assign parentage.

278

279 *Derivation of pedigree f and multilocus heterozygosity*

280 Based on the final pedigree, which incorporated information on putative relationships among
281 founders as described above, pedigree inbreeding coefficients (f_{ped}) were calculated for all
282 individuals using the R package pedantics (Morrissey, 2014). However, subsequent analyses
283 involving f_{ped} were based only on individuals with all four grandparents assigned. From the
284 microsatellite data, we also quantified each individual's standardised multilocus
285 heterozygosity (sMLH) using inbreedR (Stoffel et al., 2016). The same program was also
286 used to calculate g_2 , a quantity that estimates identity disequilibrium (the extent to which
287 heterozygosities are correlated across loci) following David, Pujol, Viard, Castella, & Goudet
288 (2007). We also used inbreedR to calculate the 95% confidence interval of g_2 by

289 bootstrapping over individuals and to permute the genetic data to generate a p -value for the
290 null hypothesis of no variance in inbreeding in the sample (i.e. $g_2 = 0$) as described in detail
291 by Stoffel et al. (2016).

292

293 *Testing for parentage assignment biases in our pedigree*

294 The majority of accepted parental relationships had very high confidence (89% at $\geq 99\%$
295 confidence). Nevertheless, Wang (2010) showed that parentage analyses can potentially be
296 biased in favour of heterozygotes, which could potentially create an artefactual positive
297 relationship between sMLH and reproductive success. We evaluated whether such a bias
298 could affect our pedigree by testing for an association between parental heterozygosity and the
299 confidence with which parents were assigned in our pedigree using a generalised linear model
300 (GLM) with a binomial error structure. A slight but statistically significant bias was found in
301 the direction of homozygotes being assigned parentage with slightly greater confidence than
302 heterozygotes (Supplementary table S1). To explore this further, we simulated pedigrees
303 based on the empirical allele frequencies of our study population. Our methods and results
304 are described in detail in the supplementary information. Briefly, initial simulations assuming
305 random mating assigned 94% of parents with a probability of 1.0 and therefore no bias could
306 be detected. Hence, we simulated an arguably more realistic pedigree with close inbreeding
307 for which parentage analysis should be technically more challenging due to high relatedness
308 among the candidate parents. Consistent with results from our empirical dataset, we found
309 that homozygotes had a slightly higher probability of being assigned parentage
310 (Supplementary table S2). Taken together, these findings suggest that any bias in our
311 pedigree should be both small and in the opposite direction to that predicted, and is therefore
312 unlikely to generate a false signal of inbreeding depression.

313

314 *Statistical analyses*

315 Strong inbreeding depression early in life will tend to deplete the adult population of inbred
316 individuals and thereby reduce the power to detect inbreeding effects later in life (Huisman et
317 al., 2016). To evaluate this possibility, we grouped individuals into six cohorts based on their
318 survival to a given age (< one, one, two, three, four or \geq five years old) and used Levene's test
319 to assess the equality of variances of f_{ped} and sMLH among the cohorts and Spearman's rank
320 to test for a decrease in mean inbreeding with increasing age. We then investigated
321 inbreeding depression for four main fitness components: (i) survival to nutritional
322 independence; (ii) survival beyond nutritional independence; (iii) yearling body mass; and (iv)
323 annual reproductive success (see below for further details). These fitness components were
324 used as response variables in four separate analyses conducted within R version 3.2.3 (R Core
325 Team, 2014). Beforehand, all of the explanatory variables were checked for collinearity using
326 pair plots and by calculating pairwise correlation coefficients. Graham (2003) showed that
327 correlations between explanatory variables as low as 0.28 may compromise model
328 parameterisation but collinearity in our models was well below this, except for f_{ped} and sMLH,
329 which we dealt with as described below. All of our models were also validated through visual
330 inspection of histograms of residuals and plots of residuals against fitted values for each of the
331 explanatory variables as recommend by Zuur, Ieno, & Saveliev (2009).

332

333 For each analysis, we constructed a set of competing models, each incorporating prior
334 knowledge of the banded mongoose system, and quantified their relative support using AIC_c-
335 weights within a multi-model inference framework. As support for a model increases, its
336 AIC_c-weight tends towards 1. To quantify the contributions of individual predictor variables,

337 we then calculated predictor-AIC_c-weights by summing the AIC_c-weights of all models
338 containing that predictor. We also followed the recommendation of Richards et al. (2011) and
339 discarded models with better supported models nested within them (i.e. models that are more
340 complicated versions of a better supported model).

341

342 Within the above framework, f_{ped} and sMLH were used as predictor variables to quantify the
343 effects of inbreeding on fitness. Including f_{ped} and sMLH in the same models is likely to
344 cause problems due to multi-collinearity because both are estimates of IBD_g. Therefore, we
345 quantified any potential effects of sMLH above and beyond f_{ped} by constructing a set of
346 models containing both f_{ped} and the residuals of sMLH on f_{ped} (henceforth termed residual
347 sMLH). As there is no statistical collinearity between f_{ped} and residual sMLH, we were able
348 to include information from the pedigree and molecular markers simultaneously without
349 biasing the regression parameter estimates (Graham, 2003). Residual sMLH can be
350 interpreted as whether an individual is more or less heterozygous than expected given their f_{ped}
351 and its effect size can be interpreted as its effect additional to that already made through its
352 relationship with f_{ped} as any variance explained by both terms is attributed to f_{ped} . This
353 technique is called sequential regression and performs well across a range of complex
354 functional relationships and collinearity structures (Dormann et al., 2013). Additional non-
355 genetic explanatory variables were analysed based on prior knowledge of the mongoose
356 system as described below.

357

358 *(i) Survival to nutritional independence*

359 As mortality is highest in banded mongooses prior to nutritional independence around day 90,
360 we first analysed survival to 90 days. A recent study found that offspring of extra group

361 matings, which tend to be more heterozygous, have higher survivorship to 90 days (Nichols,
362 Cant, & Sanderson, 2015), suggesting that there could be a direct link between inbreeding and
363 early survivorship. In the current study, data were available for a total of 489 individuals with
364 all four grandparents assigned. Survival was analysed as a binomial response variable (coded
365 as 1 = survived, 0 = died) within generalized linear mixed models (GLMMs) using lme4
366 (Bates, Maechler, Bolker, & Walker, 2015) with litter nested within pack as random effects.
367 A total of 19 competing models were constructed (see Table 1), each containing different
368 combinations of predictor variables representing plausible hypotheses to be evaluated within a
369 multi-model inference framework. We included rainfall during the 30 days prior to birth as a
370 predictor variable in all of the models, as this is robustly associated with early life survival
371 (Nichols et al., 2015; Sanderson et al., 2015). As escorting has a highly significant effect on
372 survival to 60 days (Gilchrist, 2004) but is only weakly associated with survival to 90 days
373 (Hodge, 2005), we also included escorting as a continuous variable (see above) in a subset of
374 the models. To further test for an interaction between inbreeding and stress, we constructed a
375 further subset of models containing interactions between rainfall and one of the inbreeding
376 terms (i.e. $\text{rain} * f_{\text{ped}}$ or $\text{rain} * \text{sMLH}$). As explained above, the effect of residual
377 heterozygosity was evaluated by constructing models containing both f_{ped} and residual sMLH.
378

379 *(ii) Survival beyond nutritional independence*

380 We investigated inbreeding depression for longevity based on all individuals that survived
381 beyond 90 days ($n = 428$ mongooses with at least all four grandparents in the pedigree).
382 Lifespan was investigated using Cox-proportional-hazard models in the survival package
383 (Therneau & Grambsch, 2000). Individuals that survived until the end of the study or that
384 emigrated from the study population were classified as right censored in the models. To

385 account for the non-independence of individuals within social groups, we fitted pack as a
386 frailty term, equivalent to a random effect. We also verified that the proportional hazard was
387 independent of time using plots of the scaled Schoenfeld residuals. We constructed 14
388 competing models (see table 2), all of which contained sex (coded as female = 0, male =1)
389 because males tend to have a longer lifespan (Cant, Nichols, Thompson, & Vitikainen, 2016).
390 We used mean monthly rainfall in the first year of life as a predictor variable in a subset of
391 models because it is associated with prey abundance and thereby influences lifespan (Marshall
392 et al., 2017). As described above for the models of survival to nutritional independence, we
393 also tested for an interaction between inbreeding and stress by constructing models containing
394 interactions between rainfall and the inbreeding terms.

395

396 *(iii) Yearling body mass*

397 We next investigated inbreeding depression for body mass (measured in g) at one year of age.
398 Heavier banded mongoose females breed earlier (Hodge, 2005) and may thus have higher
399 lifetime reproductive success. Also, yearling body mass exhibits inbreeding depression
400 (Sanderson et al., 2015) although the study in question did not analyse microsatellite
401 heterozygosity. Individuals were habituated to step onto a portable weighing balance for a
402 small reward of milk, which allowed us to measure body mass. Yearling body mass was
403 calculated as the average of all morning mass measurements for an individual taken between
404 350 and 380 days of age. Measurements were taken in the morning to standardise against
405 fluctuations in body mass that may occur during the day. Data on yearling body mass were
406 available for a total of 156 individuals with all four grandparents known. We constructed 53
407 competing models (See table 3) with litter nested within pack as random effects. These
408 models were run in the glmmADMB package (Fournier, Skaug, Ancheta, & Ianelli, 2012)

409 with a Gaussian error distribution. We included sex in a subset of models and rainfall in the
410 30 days prior to birth in a subset of the models as this was previously found to be positively
411 associated with body mass in one study (Nichols et al., 2015) but not in another (Sanderson et
412 al. 2015). To test for interactions between inbreeding and stress, some of these models also
413 included interactions between rainfall and the inbreeding terms. Escorting was included in a
414 further subset of models as it correlates positively with pup weight at 84 days (Hodge, 2005;
415 but see Gilchrist, 2004).

416

417 *(iv) Annual reproductive success*

418 Reproductive success is closely linked to fitness but no studies of banded mongooses have
419 previously investigated inbreeding depression for this trait. We therefore used the pedigree to
420 quantify annual reproductive success, expressed as the number of pups assigned to each
421 individual, for all animals over six months of age who survived a given year. Because
422 reproductive opportunities differ between the sexes, with most females breeding regularly
423 while male reproductive success is strongly skewed towards the oldest 3–5 males in a pack
424 (Nichols, Amos, Cant, Bell, & Hodge, 2010), separate models were constructed for each sex.
425 These were based on a total of 240 annual observations of 99 females and 354 annual
426 observations of 129 males. Annual reproductive success was modelled using a negative
427 binomial error distribution with zero-inflation within the R package glmmADMB (Skaug,
428 Fournier, Nielsen, & Magnusson, 2013). To account for multiple observations of individuals
429 and packs, we fitted individual and pack as random effects. We constructed 14 competing
430 models separately for females and males (see Tables 4a and 4b respectively). As reproductive
431 success tends to increase with age before tailing off later in life (Sanderson et al., 2015), we
432 included age and age² as predictor variables in all of the models. Average monthly rainfall

433 over the year was also included in a subset of models as a proxy for environmental stress,
434 while inbreeding–stress interactions were investigated through the inclusion of models
435 containing interactions between rainfall and the inbreeding terms.
436

For Review Only

437 **Results**

438

439 We augmented an existing microsatellite dataset comprising 1,748 individuals genotyped at
440 35–43 microsatellite loci (Sanderson et al., 2015) by genotyping an additional 192 individuals
441 at 35 microsatellites. This allowed us to enlarge the nine-generation deep banded mongoose
442 pedigree of Sanderson et al. (2015) by increasing the number of maternal links from 1,570 to
443 1,725 and the number of paternal links from 1,476 to 1,625. The restricted dataset of
444 individuals with all four grandparents assigned, which formed the basis of all subsequent
445 analyses, increased from 672 to 777.

446

447 *Inbreeding and heterozygosity*

448 Our pedigree uncovered appreciable variance in inbreeding (mean $f_{\text{ped}} = 0.058$, variance =
449 0.006), with the majority of individuals (66.4%) being to some extent inbred (Figure 1, top
450 marginal histogram). Weak inbreeding ($0 < f_{\text{ped}} < 0.125$) accounted for 46.5% of the
451 population, while 12.9% of individuals were moderately inbred ($0.125 \leq f_{\text{ped}} < 0.25$) and 7.1%
452 were closely inbred ($f_{\text{ped}} \geq 0.25$). Microsatellite heterozygosity (sMLH) was approximately
453 normally distributed with a mean of 0.982 and a variance of 0.034 (Figure 1, right marginal
454 histogram) and correlated significantly with f_{ped} ($R = -0.34$, $p < 0.001$). Furthermore, the
455 measure g_2 , which quantifies the extent to which heterozygosity is correlated across loci, was
456 positive (0.012, 95% CI = 0.007–0.018) indicating that the microsatellites are capturing
457 variation in inbreeding. As observed in other species (e.g. Huisman et al. 2016), appreciable
458 variation was observed in sMLH among individuals with the same f_{ped} .

459

460 *Changes in inbreeding with age*

461 If inbred individuals experience stronger viability selection early in life, the variance in
462 inbreeding should be lower in adults, making it more difficult to detect inbreeding depression
463 for late-acting traits (Huisman et al., 2016). To investigate this possibility, we divided the
464 mongooses into six cohorts based on their survival to a given age (see Materials and methods)
465 and tested for differences in the variance of f_{ped} and sMLH among these cohorts using
466 Levene's tests. Neither of the inbreeding measures showed a decrease in variance with age
467 (table S3) and the variance in sMLH did not differ significantly among cohorts ($F_5 = 0.74$, $p =$
468 0.59). However, the cohorts did not have equal variance in f_{ped} ($F_5 = 2.36$, $p = 0.03$). This
469 result appears to be driven by low sampling variance in individuals who survived between one
470 and two years as the variance in f_{ped} no longer differed significantly among cohorts after these
471 animals were excluded from the analysis. Taken together, these findings suggest that viability
472 selection against inbred individuals does not reduce the variance in inbreeding with age. In
473 line with this, we also found no evidence for a decline in the mean level of inbreeding with
474 increasing age (f_{ped} $\rho = 0.043$, $p = 0.23$; sMLH $\rho = -0.01$, $p = 0.78$; table S3).

475

476 *Survival to nutritional independence*

477 We found that the model of survival to nutritional independence with the greatest AIC_c
478 support included rainfall in the 30 days prior to birth and escorting as fixed effect explanatory
479 variables (Table 1, intercept = -0.5364 ± 0.4514 SE, rainfall $\beta = 0.3577 \pm 0.1348$ SE,
480 escorting $\beta = 0.8764 \pm 0.4084$ SE, random effects: pack SD = 0.000, litter nested within pack
481 SD = 1.57). The second and third most supported models included rain and escorting as well
482 as an inbreeding term (Table 1). However, as they had the best model nested within them (i.e.
483 they were more complex but less supported versions of the first model) we did not consider
484 them further, as recommended by Richards et al. (2011).

485

486 *Survival beyond nutritional independence*

487 The results of our analysis of adult survival were equivocal (Table 2). The highest ranking
488 model included sMLH but had roughly equivalent AIC_c support ($\Delta\text{AIC}_c < 1$) to a simple
489 model that included only sex. As AIC_c tends to slightly favour complex models, especially
490 when there is uncertainty over the best model (Symonds & Moussalli, 2011), our results do
491 not provide convincing evidence of inbreeding depression for longevity.

492

493 *Yearling body mass*

494 By contrast, strong support was found for inbreeding depression in yearling body mass, with
495 all of the top 12 models containing f_{ped} as a fixed effect explanatory variable (Table 3) and the
496 predictor-AIC_c-weight for f_{ped} being high at 0.96. The top ranking model contained sex and
497 f_{ped} (Table 3, Figure 2; intercept = 1162 ± 53 SE, sex $\beta = 59 \pm 19$ SE $f_{\text{ped}} \beta = -382 \pm 127$ SE,
498 random effects: pack SD = 125.5, litter nested within pack SD = 37.6). As before, we
499 disregarded less supported models with this model nested within them as suggested by
500 Richards et al. (2011).

501

502 *Annual reproductive success*

503 Focusing first on female reproductive success, the top ranking model contained age + age² +
504 f_{ped} but the next best model had very similar AIC_c support but did not contain f_{ped} (Table 4a).
505 Because AIC_c support for these two models was so similar and AIC exhibits a slight
506 preference for overly complex models, the simpler model should be preferred. Consequently,
507 our data provided only limited support for inbreeding depression for female annual
508 reproductive success as our preferred model contained only age and age² (intercept = -1.2539

509 ± 0.3773 SE, age $\beta = 0.7616 \pm 0.1776$ SE, age² $\beta = -0.0480 \pm 0.0244$ SE). By contrast, the
510 best supported model for males contained both f_{ped} and residual sMLH (intercept = $-2.9481 \pm$
511 0.4792 SE, age $\beta = 1.4452 \pm 0.1905$ SE, age² $\beta = -0.1343 \pm 0.0209$ SE, f_{ped} $\beta = -6.2994 \pm$
512 1.7203 SE, residual sMLH $\beta = 2.0920 \pm 0.7646$ SE). This not only provides evidence for
513 inbreeding depression for male annual reproductive success, but also suggests that marker
514 heterozygosity captures a significant amount of variance that is not explained by f_{ped} . This
515 model was nested within the second and third highest ranking models, which also had
516 considerable AIC_c support and respectively contained rain and an interaction between rain and
517 f_{ped} .

518
519 Consistent with theoretical expectations, the best supported model of annual male
520 reproductive success revealed a negative association with f_{ped} (Figure 3a) and a positive
521 association with residual sMLH (Figure 3b). Inbred males with an f_{ped} value of 0.25 were
522 predicted by the model to have approximately 79% fewer offspring than fully outbred
523 individuals with an f_{ped} value of zero, while males with residual sMLH values one standard
524 deviation above zero (0.185) were predicted to have 47% more offspring than individuals with
525 residual sMLH equal to zero. This indicates that within f_{ped} classes, relatively heterozygous
526 individuals tend to have greater reproductive fitness.

527

528 *Effect sizes of the inbreeding terms*

529 To provide further insights into the effect sizes of the inbreeding terms, we constructed three
530 alternative models separately for each fitness trait. These models contained non-inbreeding
531 terms that were retained in the top ranking models described above for each trait, while in
532 addition the first model contained f_{ped} , the second contained sMLH and the third contained f_{ped}

533 plus residual sMLH. To evaluate inbreeding effects, we then calculated effect sizes and their
534 corresponding 95% confidence intervals (CIs) for all of the predictor variables contained in
535 each model. The results are summarised separately for each trait in Figure 4. Consistent with
536 results from the information theoretic approach, the 95% CIs of the effect sizes of all three
537 inbreeding terms overlapped zero for survival to nutritional independence, survival beyond
538 nutritional independence and female reproductive success (Figure 4a, b and d), suggesting that
539 there is very little evidence for inbreeding depression for these traits. Also as expected, f_{ped}
540 had negative point estimates whose corresponding 95% CIs did not overlap zero in models of
541 yearling body mass and annual male reproductive success (Figure 4c and e), while sMLH and
542 residual sMLH only had positive estimates and 95% CIs not overlapping zero in models of
543 male reproductive success (Figure 4e).

544

545 *Associated p - and R^2 values*

546 In order to evaluate the sensitivity of our results to the statistical framework employed, we
547 determined the statistical significance of f_{ped} , sMLH and residual sMLH using a frequentist
548 approach. Separately for each trait, we derived p -values for each of the inbreeding terms
549 using likelihood ratio tests. The significance of f_{ped} and sMLH was derived by comparing
550 models containing these terms with equivalent 'null models' containing only the relevant non-
551 inbreeding terms, while p -values for residual sMLH were obtained through the comparison of
552 models containing f_{ped} plus residual sMLH with equivalent models containing only f_{ped} . To
553 provide an indication of the proportion of variance explained by each model, we also
554 calculated conditional R^2 values for GLMMs (Nakagawa & Schielzeth, 2013) and Cox and
555 Snell's pseudo R^2 values for Cox proportional hazard models (Cox & Snell, 1989). However,
556 this was not possible for zero-inflated negative binomial GLMMs so we instead report log

557 likelihood values for these models (Table 5). To allow direct comparison with other studies,
558 correlation coefficients between the two inbreeding measures and each fitness trait are also
559 provided in the supporting information (table S4). Consistent with the results of the multi-
560 model approach described above, we found a highly significant effect of f_{ped} on yearling body
561 mass, which explained almost 5% of the total variation (Table 5c), although sMLH did not
562 explain a significant amount of variance in this trait. By contrast, both f_{ped} and sMLH
563 explained significant variation in male annual reproductive success (Table 5e). Furthermore,
564 adding residual sMLH to a model containing only f_{ped} resulted in a significant improvement to
565 the model of annual male reproductive success ($p = 0.007$, Table 5e), suggesting that for some
566 traits genetic markers may capture variation in inbreeding above and beyond that explained by
567 f_{ped} .

568

569 *Sensitivity to marker number*

570 To further investigate the explanatory power of f_{ped} and marker heterozygosity, we directly
571 compared three of our models of annual male reproductive success in which the inbreeding
572 terms were f_{ped} (M4 in table 4b), sMLH (M3 in table 4b) and f_{ped} plus residual sMLH (M8 in
573 table 4b) respectively, and explored the sensitivity of model AIC_c to marker number. As
574 expected, AIC_c decreased steadily with increasing marker number (Figure 5). With fewer
575 than around 20 markers, sMLH did not perform as well as f_{ped} , but with 30–40 markers AIC_c
576 values for the two models were very similar. Furthermore, the model containing both f_{ped} and
577 residual sMLH became increasingly superior to the model containing only f_{ped} as more
578 markers were deployed.

579

580 *Testing for local effects*

581 Finally, we tested for the possible involvement of local effects involving specific
582 microsatellite loci by adapting the approach of Szulkin, Bierne, & David (2010). Specifically,
583 we compared a model of male reproductive success containing age, age^2 , f_{ped} and residual
584 sMLH with a model in which residual sMLH was replaced by separate terms for the residual
585 heterozygosity of each of the microsatellite loci. The second model was not a significant
586 improvement over the first, although the corresponding p -value was close to significance (-
587 $2LL_{30} = 42.06$, $p = 0.07$). Our results are therefore more consistent with inbreeding
588 depression than with a mechanism based on one or a small number of local effects.
589

For Review Only

590 **Discussion**

591

592 Although inbreeding depression is known to be important in many wild populations, relatively
593 few studies are large and detailed enough either to compare multiple traits at different stages
594 in the life cycle or to investigate the relative explanatory power of pedigree-based and
595 molecular estimates of inbreeding. We therefore used an exceptionally comprehensive long-
596 term study of banded mongooses both to quantify inbreeding depression for early and late-
597 acting traits and to evaluate the hypothesis that marker heterozygosity may capture fitness
598 variation above and beyond that explained by f_{ped} . Contrary to our initial expectations, we did
599 not find evidence for strong viability selection against inbred individuals early in life, but
600 instead detected inbreeding depression for traits relating to individual quality (i.e. yearling
601 body mass and male annual reproductive success). Furthermore, we found that fitting f_{ped} and
602 residual sMLH together in a single model explained significantly more of the variance in male
603 annual reproductive success than using f_{ped} alone. However this was not the case for yearling
604 body mass, where f_{ped} explained variation in fitness but sMLH did not.

605

606 *Inbreeding depression for different traits*

607 Theory predicts that inbreeding depression should be greatest for traits closely linked to
608 fitness because traits under strong directional selection will exhibit greater directional
609 dominance (Lynch & Walsh, 1998). This is supported by a meta-analysis that found stronger
610 inbreeding depression for life history traits such as survival and fecundity than for
611 morphological traits such as body weight (DeRose & Roff, 1999). Given that all of the traits
612 we analysed in banded mongooses are arguably very closely linked to fitness, we were
613 initially surprised not to find inbreeding depression for either survival to nutritional

614 independence or longevity. One potential explanation for this is that inbreeding depression
615 for early survival could be buffered by the social system of this species (Ihle, Hutter, &
616 Tschirren, 2017; Nielsen et al., 2012; Pilakouta, Jamieson, Moorad, & Smiseth, 2015)
617 especially if escorts preferentially direct care towards inbred individuals (Thünken, Bakker,
618 Baldauf, & Kullmann, 2007). However, due to the complexity of the banded mongoose
619 system, testing this hypothesis lies beyond the scope of the current study. Alternatively, as
620 the environment is relatively benign and major causes of death in our study population are
621 predation and injuries sustained during aggressive interactions between social groups (Cant et
622 al., 2013), there may be relatively little scope for strong genetic effects on survival. A further
623 possibility is that our study may have lacked the statistical power to detect inbreeding
624 depression for traits with smaller available sample sizes, such as female annual reproductive
625 success. However, this seems unlikely to account for the absence of detectable inbreeding
626 depression for early-acting traits like survival to nutritional independence as sample sizes for
627 these analyses were more than double what was available for yearling body mass, where
628 inbreeding depression was detected. Nevertheless, we cannot discount the possibility that
629 inbreeding depression might influence survival at an even earlier stage of development, for
630 instance *in utero* or during their first month *post partum* before emergence from the
631 underground den.

632

633 As several studies have shown that inbreeding depression can be magnified by stress
634 (Armbruster & Reed, 2005; Fox & Reed, 2011; Meagher, Penn, & Potts, 2000; Reed, Fox,
635 Enders, & Kristensen, 2012, Noren et al 2016), we included interactions between rainfall and
636 both measures of inbreeding in all of our analyses as rainfall is a proxy for food availability.
637 We found that none of the top ranking models of survival to nutritional independence,

638 longevity, yearling body mass or annual reproductive success contained interactions between
639 rainfall and either f_{ped} or sMLH. Furthermore, although rainfall has a strong effect on survival
640 to nutritional independence (Nichols et al., 2015; Sanderson et al., 2015) and was therefore
641 included as a main effect in all models of this particular trait, rainfall did not feature in any of
642 the chosen models of the other three fitness traits. Thus, our rainfall measures do not appear
643 to strongly influence most of the investigated traits, which may help to explain why
644 interactions involving rainfall were not found.

645

646 Alternatively, social stressors might be disproportionately important in this cooperative
647 breeding species. Consistent with this, strong inbreeding depression was found for male
648 annual reproductive success, with closely inbred individuals ($f_{\text{ped}} \geq 0.25$) having 79% lower
649 annual reproductive success than individuals with an f_{ped} of zero, whereas our results for
650 female reproductive success provided at best limited support for inbreeding depression.
651 Although the sample size of female observations was smaller, sex-specific inbreeding
652 depression would be consistent with previous studies of wild mice showing that male-male
653 competition amplifies inbreeding depression (Meagher et al., 2000). It would also be in line
654 with stronger reproductive skew in male versus female banded mongooses (Nichols et al.,
655 2010) as stronger directional selection is expected to increase inbreeding depression.

656

657 *Detecting inbreeding depression with pedigrees and genetic markers*

658 Pedigrees have for many years been the gold standard for quantifying inbreeding depression
659 in wild populations (Pemberton, 2004, 2008). However, pedigree data are often incomplete
660 and assignment errors can introduce significant error into the estimation of f_{ped} (Reid et al.,
661 2014) while the assumption that the founders are outbred and unrelated to one another may

662 also be violated in closed or structured populations. In addition, f_{ped} is a measure of the
663 expected IBD_g of an individual based on its pedigree and cannot capture stochastic variation
664 in realised IBD_g resulting from Mendelian segregation (Hedrick et al., 2016; Hill & Weir,
665 2011; Knief et al., 2016). Consequently, there has been growing interest in the extent to
666 which f_{ped} and marker heterozygosity can capture inbreeding effects, either independently or
667 when analysed together, as well as in how the explanatory power of genetic markers varies
668 with the number of loci that can be genotyped.

669

670 Several studies have compared the ability of pedigrees and microsatellites to detect inbreeding
671 depression. These have reached the general consensus that f_{ped} usually performs better (e.g.
672 Ólafsdóttir & Kristjánsson, 2008; Slate et al., 2004; Taylor et al., 2010), even when hundreds
673 of microsatellites are used (Nietlisbach et al., 2017), although it is also to be expected that
674 tens of thousands of SNPs will outperform f_{ped} (Huisman et al., 2016; Kardos et al., 2015).
675 Nevertheless, both Forstmeier et al. (2012) and Hammerly et al. (2013) detected stronger
676 inbreeding effects with around ten microsatellites than with f_{ped} . Our results fall somewhere
677 in between these opposite ends of the spectrum, with heterozygosity based on around 40
678 microsatellites having roughly equivalent explanatory power to f_{ped} for male annual
679 reproductive success but not for yearling body mass. This probably reflects a variety of
680 factors as discussed below.

681

682 First, most pedigrees suffer to a greater or lesser extent from errors in the assignment of
683 parental relationships, which can lead to significant and often downward bias in the estimation
684 of inbreeding depression (Reid et al., 2014). This could partly explain the contrasting results
685 of Nietlisbach et al. (2017) and Hammerly et al. (2013), as the former study was able to

686 genotype the parents of all of the individuals used in the analysis for a very large number of
687 microsatellites, resulting in an unusually accurate pedigree, whereas Hammerly et al. (2013)
688 recognised that their pedigree contained a significant number of errors. Although it is difficult
689 to directly compare different studies, our banded mongoose pedigree probably sits closer to
690 the song sparrow end of the continuum, as our panel of microsatellites was moderately large
691 and the majority of the adult population (all but four parents, Sanderson et al. 2015) was
692 included.

693

694 A second factor that may influence the relative explanatory power of pedigrees and genetic
695 markers is pedigree depth. Pedigree-based inbreeding estimates become increasingly accurate
696 with increasing depth, although these estimates become only marginally more precise beyond
697 five generations in populations with certain structures (Slate et al., 2004, Kardos et al., 2015).
698 Therefore, deeper pedigrees will tend to capture more of the variance in IBD_g within a given
699 population and leave less 'undetected inbreeding' for the markers to capture (Nietlisbach et al.,
700 2017). This could potentially help to explain why residual heterozygosity accounts for
701 additional fitness variation in one of the two traits that showed inbreeding depression in our
702 study, as 54% of individuals in the song sparrow pedigree had eight or more known ancestral
703 generations, whereas our equivalent value was only 3% and around half of all individuals in
704 our banded mongoose pedigree had fewer than five generations known.

705

706 Third, the information content of the genetic markers used in a study will influence how well
707 heterozygosity measures inbreeding. Homozygosity measured at genetic markers with few
708 alleles and/or highly skewed allele frequencies is more likely by chance to reflect IBS than
709 IBD and so may provide relatively little information about an individual's level of inbreeding.

710 Calculating the IBD–IBS discrepancy for our dataset following Knief et al. (2016) resulted in
711 an estimate of 49%. This is higher than in zebra finches (13%, Knief et al., 2016) and may in
712 part reflect the relatively low allelic richness of our microsatellites (average number of alleles
713 = 5.2, Supplementary table S5). However, this does not appear to have been a major issue for
714 our study, probably due to the relatively large panel of available microsatellites. It might be
715 interesting to explore this further in future studies by attempting to develop 'ideal markers'
716 where there is little to no IBD–IBS discrepancy. One possible strategy would be to genotype
717 small panels of SNPs residing within known runs of homozygosity (ROH) following the
718 suggestion of Knief et al. (2016).

719

720 In addition, factors intrinsic to a given system may also play a role, such as the frequency of
721 close inbreeding, the number of chromosomes and genetic map length. For example,
722 theoretical work by Hill & Weir (2011) and simulations by Hedrick et al. (2016) suggest that
723 the variation in realised IBD_g around that expected by f_{ped} will be greater for closer
724 inbreeding, and hence that the type and variance of inbreeding in a population will affect how
725 well f_{ped} estimates IBD_g . We know that close inbreeding is relatively common in banded
726 mongooses, not because of small population sizes but because both sexes frequently remain in
727 their natal group for their entire lives and breed with other group members (Nichols et al.,
728 2014). Hence, the relatively high frequency of close inbreeding in this species could
729 potentially help to explain our results.

730

731 Furthermore, f_{ped} will be relatively imprecise in species with fewer chromosomes and shorter
732 genetic maps because genomes inherited in larger blocks will exhibit greater variance in
733 realised IBD_g for a given value of f_{ped} (Franklin, 1977; Hill & Weir, 2011; Kardos et al., 2015;

734 Stam, 1980). Genomes inherited in larger blocks should therefore provide greater scope to
735 detect inbreeding depression with relatively few molecular markers (Forstmeier et al., 2012).
736 The size of these blocks is partly determined by the number of chromosomes because the
737 proportion of unlinked loci will increase with chromosome number (Weir, Avery, & Hill,
738 1980), while within chromosomes both the number and distribution of crossovers will play a
739 role (Knief et al., 2016). To illustrate this point, nearly a third of the zebra finches genome
740 segregates in only four blocks because almost half of the autosomal genome comprises four
741 chromosomes that experience very little recombination (Forstmeier et al., 2012). It is
742 currently difficult for us to judge how these factors could have influenced our results as the
743 number of chromosomes in banded mongoose is neither small nor large ($2n = 36$, Fredga,
744 1972) and the recombination landscape of this species has not yet been characterised.

745

746 Factors that influence the relative ability of f_{ped} and markers to detect inbreeding depression
747 will also vary among populations and are expected to differ systematically between large
748 populations and smaller, threatened ones. Small or fragmented populations often have higher
749 rates of inbreeding and lower genetic diversity and Grueber, Wallis, & Jamieson (2008) argue
750 that these and other differences make it difficult to generalise results from outbred populations
751 to threatened ones. It is therefore worth considering how similar systems are in the
752 prevalence of inbreeding before extrapolating results between them. Furthermore, historical
753 changes in the structure of a population, including bottlenecks and population admixture, may
754 also create variance in inbreeding *sensu lato* (Bierne, Tsitrone, & David, 2000; Grueber et al.,
755 2008; Weir et al., 1980). Consequently, the number of markers needed to accurately quantify
756 IBD_g will also depend on the demographic history of the population in question (Miller et al.,
757 2014).

758

759 *Capturing inbreeding depression with sequential regression*

760 Although pedigrees clearly fail to capture variation in heterozygosity about the genome-wide
761 expectation given by f_{ped} , relatively few studies have attempted to quantify the amount of
762 fitness variation that genetic markers might capture additional to that explained by f_{ped} . Some
763 studies approached this question by fitting f_{ped} and heterozygosity as predictor variables in the
764 same statistical models of the focal traits (e.g. Bensch et al., 2006; Grueber et al., 2011,
765 Nietlisbach et al. 2017). However, this approach may be problematic because heterozygosity
766 is often correlated with f_{ped} and including collinear variables in a model can lead to inaccurate
767 parameter estimates (Graham, 2003). We therefore used sequential regression as an
768 alternative approach that attributes all of the shared variance to f_{ped} and is therefore able to
769 estimate how well marker heterozygosity explains variation in fitness after controlling for f_{ped}
770 without biasing parameter estimates. Using an information theoretic approach, we found that
771 the best model of male annual reproductive success contained residual sMLH as well as f_{ped} .
772 This was also supported by a frequentist approach, which uncovered a highly significant ($p =$
773 0.007) effect of residual sMLH. By contrast, residual sMLH did not explain significant
774 variation in yearling weight. One potential explanation for this could be that male
775 reproductive success exhibits stronger inbreeding depression, which may make residual
776 heterozygosity effects easier to detect.

777

778 An alternative to controlling statistically for f_{ped} is to control for this experimentally by
779 screening genetic markers in individuals chosen to have the same f_{ped} . For example,
780 Hemmings et al. (2012) used 384 genome-wide distributed SNPs to estimate homozygosity in
781 zebra finches with the same f_{ped} , finding that the most homozygous birds were less likely to

782 survive to sexual maturity. This study echoes an earlier paper where full-sibling reed warblers
783 were compared (Hansson, Bensch, Hasselquist, & Åkesson, 2001) and where again
784 heterozygosity correlated with fitness despite identical f_{ped} . A key difference is that Hansson
785 et al. (2001) used five microsatellites, leading the authors to conclude that a local effect was
786 responsible, whereas the much larger panel used by Hemmings et al. (2012) more or less
787 precludes a dominant role for only one or two loci. Consistent with the latter study, two lines
788 of evidence are suggestive of a genome-wide mechanism in banded mongooses. First, in our
789 models of annual male reproductive success, we found that AIC_c steadily fell as the number of
790 randomly sampled microsatellite loci increased, regardless of whether sMLH or residual
791 sMLH were fitted as predictor variables. Second, we did not find that a model incorporating
792 the single-locus heterozygosities of all of the loci explained significantly more variation than a
793 model containing only sMLH. Although the second test is admittedly conservative,
794 collectively our results point towards a polygenic architecture, consistent with the widespread
795 view that the majority of inbreeding effects are caused by many loci with small effect sizes
796 distributed across the genome (Charlesworth & Willis, 2009; Szulkin et al., 2010).

797

798 *Future perspectives*

799 Looking to the future, although ours and many other studies have quantified heterozygosity
800 using microsatellites, simulations clearly indicate that tens of thousands of markers will
801 outperform even very deep pedigrees at capturing inbreeding depression, particularly when
802 they can be mapped to a reference genome to quantify ROH (Kardos et al., 2015; Wang,
803 2016). This is supported by a growing number of empirical studies of wild populations using
804 approaches like restriction site associated DNA sequencing (Hoffman et al., 2014), high
805 density SNP arrays (Chen et al., 2016; Huisman et al., 2016) and whole-genome resequencing

806 (Kardos et al., 2018). As the costs of these and related methods continue to fall, they are
807 likely to become preferred approaches for studying inbreeding and its consequences in wild
808 populations.

809

810 *Conclusion*

811 We used a high quality pedigree together with data from up to 43 microsatellites to investigate
812 inbreeding depression in a cooperatively breeding species where mating between close
813 relatives is common. We detected inbreeding depression for yearling body weight and annual
814 male reproductive success but found no evidence for inbreeding affecting survival, either to
815 nutritional independence or beyond. Furthermore, for one out of the two traits exhibiting
816 inbreeding depression, our panel of microsatellites had similar explanatory power to f_{ped} and
817 residual sMLH explained a significant proportion of fitness variation when fitted in a model
818 together with f_{ped} . Our findings therefore suggest that, at least under some circumstances,
819 combining pedigree and molecular measures of inbreeding may allow us to explain more
820 fitness variation and thereby improve our understanding of the genetic variance underpinning
821 fitness variation in wild populations.

822

823 **Acknowledgements**

824 The work was supported by a Deutsche Forschungsgemeinschaft (DFG) standard grant (HO
825 5122/5-1) and a dual PhD studentship from Liverpool John Moores University. We are
826 grateful to Uganda Wildlife Authority and Uganda National Council for Science and
827 Technology for permission to carry out our research and the Wardens of Queen Elizabeth
828 National Park for logistical support. We thank F. Mwanguhya, S. Kyambulima, K. Mwesige
829 and R. Businge for assistance in the field and M. Galipaud and M. Stoffel for discussions on
830 the analyses. Finally, the manuscript benefited greatly from the constructive comments of J.
831 Pemberton, M. Kardos and three anonymous reviewers.

832

833 **References**

- 834 Armbruster, P., & Reed, D. H. (2005). Inbreeding depression in benign and stressful
835 environments. *Heredity*, *95*(3), 235–242. <http://doi.org/10.1038/sj.hdy.6800721>
- 836 Balloux, F., Amos, W., & Coulson, T. (2004). Does heterozygosity estimate inbreeding in real
837 populations? *Molecular Ecology*, *13*(10), 3021–3031. <http://doi.org/10.1111/j.1365-294X.2004.02318.x>
- 839 Bates, D. M., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects
840 models using lme4. *Journal of Statistical Software*, *67*(1), 1–48.
841 <http://doi.org/10.1177/009286150103500418>
- 842 Bensch, S., Andrén, H., Hansson, B., Pedersen, H. C., Sand, H., Sejberg, D., ... Liberg, O.
843 (2006). Selection for heterozygosity gives hope to a wild population of inbred wolves.
844 *PLoS ONE*, *1*(1). <http://doi.org/10.1371/journal.pone.0000072>
- 845 Bierne, N., Tsitrone, A., & David, P. (2000). An Inbreeding Model of Associative
846 Overdominance During a Population Bottleneck. *Genetics*, *155*(4), 1981–1990.
- 847 Cant, M. A. (2000). Social control of reproduction in banded mongooses. *Animal Behaviour*,
848 *59*(1), 147–158. <http://doi.org/10.1006/anbe.1999.1279>
- 849 Cant, M. A., Nichols, H. J., Thompson, F. J., & Vitikainen, E. (2016). Banded mongooses :
850 demography , life history , and social behavior. *Cooperative Breeding in Vertebrates:
851 Studies of Ecology, Evolution, and Behavior*, 318–337.
- 852 Cant, M. A., Oтали, E., & Mwanguhya, F. (2001). Eviction and dispersal in co-operatively
853 breeding banded mongooses (*Mungos mungo*). *Journal of Zoology*, *254*(2), 155–162.
854 <http://doi.org/10.1017/S0952836901000668>
- 855 Cant, M. A., Vitikainen, E., & Nichols, H. (2013). Demography and social evolution of
856 banded mongooses. *Adv. Study Behav.*
- 857 Chapman, J. R., Nakagawa, S., Coltman, D. W., Slate, J., & Sheldon, B. C. (2009). A
858 quantitative review of heterozygosity-fitness correlations in animal populations.
859 *Molecular Ecology*, *18*(13), 2746–2765. <http://doi.org/10.1111/j.1365-294X.2009.04247.x>
- 861 Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature
862 Reviews. Genetics*, *10*(11), 783–96. <http://doi.org/10.1038/nrg2664>
- 863 Chen, N., Cosgrove, E. J., Bowman, R., Fitzpatrick, J. W., Clark, A. G., Chen, N., ... Clark,
864 A. G. (2016). Genomic Consequences of Population Decline in the Endangered Florida
865 Scrub-Jay. *Current Biology*, *26*(21), 2974–2979.
866 <http://doi.org/10.1016/j.cub.2016.08.062>
- 867 Cox, D., & Snell, E. (1989). *Analysis of binary data*. London: Chapman & Hall.
- 868 David, P., Pujol, B., Viard, F., Castella, V., & Goudet, Jerom. (2007). Reliable selfing rate
869 estimates from imperfect population genetic data. *Molecular Ecology*, *16*(12), 2474–
870 2487.
- 871 DeRose, M. A., & Roff, D. A. (1999). A Comparison of Inbreeding Depression in Life-
872 History and Morphological Traits in Animals. *Evolution*, *53*(4), 1288–1292.
- 873 Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., ... Lautenbach, S.
874 (2013). Collinearity: A review of methods to deal with it and a simulation study
875 evaluating their performance. *Ecography*, *36*(1), 027–046. <http://doi.org/10.1111/j.1600-0587.2012.07348.x>
- 877 Falconer, D. S., & Mackay, T. F. C. (1996). Introduction to quantitative genetics.
878 <http://doi.org/10.1002/bimj.19620040211>
- 879 Fisher, R. A. (1965). *The theory of inbreeding. The theory of inbreeding*. Oliver & Boyd,
880 Edinburgh and London.

- 881 Forstmeier, W., Schielzeth, H., Mueller, J. C., Ellegren, H., & Kempenaers, B. (2012).
882 Heterozygosity-fitness correlations in zebra finches: microsatellite markers can be better
883 than their reputation. *Molecular Ecology*, *21*(13), 3237–49.
884 <http://doi.org/10.1111/j.1365-294X.2012.05593.x>
- 885 Fournier, D., Skaug, H., Ancheta, J., & Ianelli, J. (2012). AD Model Builder: using automatic
886 differentiation for statistical inference of highly parameterized complex nonlinear
887 models. *Optimization Methods*.
- 888 Fox, C. W., & Reed, D. H. (2011). Inbreeding depression increases with environmental stress:
889 An experimental study and meta-analysis. *Evolution*, *65*(1), 246–258.
890 <http://doi.org/10.1111/j.1558-5646.2010.01108.x>
- 891 Franklin, I. (1977). The distribution of the proportion of the genome which is homozygous by
892 descent in inbred individuals. *Theoretical Population Biology*, *11*(1), 60–80.
- 893 Fredga, K. (1972). Comparative chromosome studies in mongooses (Carnivora, Viverridae).
894 *Hereditas*, *71*(1), 1–74. <http://doi.org/10.1111/j.1601-5223.1972.tb01005.x>
- 895 Gilchrist, J. S. (2004). Pup escorting in the communal breeding banded mongoose: behavior,
896 benefits, and maintenance. *Behavioral Ecology*, *15*(6), 952–960.
897 <http://doi.org/10.1093/beheco/arh071>
- 898 Graham, M. H. (2003). Confronting Multicollinearity in Ecological Multiple Regression,
899 *84*(11), 2809–2815. <http://doi.org/10.1890/02-3114>
- 900 Grueber, C. E., Wallis, G. P., & Jamieson, I. G. (2008). Heterozygosity-fitness correlations
901 and their relevance to studies on inbreeding depression in threatened species. *Molecular*
902 *Ecology*, *17*(18), 3978–3984. <http://doi.org/10.1111/j.1365-294X.2008.03910.x>
- 903 Grueber, C. E., Waters, J. M., & Jamieson, I. G. (2011). The imprecision of heterozygosity-
904 fitness correlations hinders the detection of inbreeding and inbreeding depression in a
905 threatened species. *Molecular Ecology*, *20*(1), 67–79. <http://doi.org/10.1111/j.1365-294X.2010.04930.x>
- 907 Hadfield, J., Richardson, D., & Burke, T. (2006). Towards unbiased parentage assignment:
908 combining genetic, behavioural and spatial data in a Bayesian framework. *Molecular*
909 *Ecology*, *15*(12), 3715–3730.
- 910 Hammerly, S. C., Morrow, M. E., & Johnson, J. A. (2013). A comparison of pedigree- and
911 DNA-based measures for identifying inbreeding depression in the critically endangered
912 Attwater's Prairie-chicken. *Molecular Ecology*, *22*(21), 5313–5328.
913 <http://doi.org/10.1111/mec.12482>
- 914 Hansson, B., Bensch, S., Hasselquist, D., & Åkesson, M. (2001). Microsatellite diversity
915 predicts recruitment of sibling great reed warblers. *Proceedings. Biological Sciences /*
916 *The Royal Society*, *268*(1473), 1287–1291. <http://doi.org/10.1098/rspb.2001.1640>
- 917 Hansson, B., Wester Dahl, H., Hasselquist, D., Åkesson, M., & Bensch, S. (2004). Does
918 Linkage Disequilibrium Generate Heterozygosity-Fitness Correlations in Great Reed
919 Warblers? *Evolution*, *58*(4), 870–879. <http://doi.org/10.1111/j.0014-3820.2004.tb00418.x>
- 921 Hedrick, P. W., & Garcia-Dorado, A. (2016). Understanding Inbreeding Depression, Purging,
922 and Genetic Rescue. *Trends in Ecology & Evolution*, *31*(12), 940–952.
923 <http://doi.org/10.1016/j.tree.2016.09.005>
- 924 Hedrick, P. W., Kardos, M., Peterson, R. O., & Vucetich, J. A. (2016). Genomic Variation of
925 Inbreeding and Ancestry in the Remaining Two Isle Royale Wolves. *Journal of Heredity*,
926 *108*(2), 120–126. <http://doi.org/10.1093/jhered/esw083>
- 927 Hemmings, N. L., Slate, J., & Birkhead, T. R. (2012). Inbreeding causes early death in a
928 passerine bird. *Nature Communications*, *3*, 863. <http://doi.org/10.1038/ncomms1870>

- 929 Hill, W. G., & Weir, B. S. (2011). Variation in actual relationship as a consequence of
930 Mendelian sampling and linkage. *Genetics Research*, 93(1), 47–64.
931 <http://doi.org/10.1017/S0016672310000480>
- 932 Hodge, S. J. (2005). Helpers benefit offspring in both the short and long-term in the
933 cooperatively breeding banded mongoose. *Proceedings of the Royal Society B*,
934 272(1580), 2479–84.
- 935 Hodge, S. J. (2007). Counting the costs: the evolution of male-biased care in the cooperatively
936 breeding banded mongoose. *Animal Behaviour*, 74(4), 911–919.
937 <http://doi.org/10.1016/j.anbehav.2006.09.024>
- 938 Hoffman, J. I., Simpson, F., David, P., Rijks, J. M., Kuiken, T., Thorne, M. A. S., ...
939 Dasmahapatra, K. K. (2014). High-throughput sequencing reveals inbreeding depression
940 in a natural population. *Proceedings of the National Academy of Sciences of the United
941 States of America*, 111(10), 3775–80. <http://doi.org/10.1073/pnas.1318945111>
- 942 Huisman, J., Kruuk, L. E. B., Ellis, P. A., Clutton-Brock, T., & Pemberton, J. M. (2016).
943 Inbreeding depression across the lifespan in a wild mammal population. *Proceedings of
944 the National Academy of Sciences*, 113(13), 3585–3590.
945 <http://doi.org/10.1073/pnas.1518046113>
- 946 Ihle, K. E., Hutter, P., & Tschirren, B. (2017). Increased prenatal maternal investment reduces
947 inbreeding depression in offspring. *Proceedings of The Royal Society B*, 284, 20171347.
948 <http://doi.org/10.1098/rspb.2017.1347>
- 949 Jones, O. R., & Wang, J. (2010). COLONY: a program for parentage and sibship inference
950 from multilocus genotype data. *Molecular Ecology Resources*, 10(3), 551–5.
951 <http://doi.org/10.1111/j.1755-0998.2009.02787.x>
- 952 Jordan, N. R., Mwanguhya, F., Kyabulima, S., Rüedi, P., & Cant, M. A. (2010). Scent
953 marking within and between groups of wild banded mongooses. *Journal of Zoology*,
954 280(1), 72–83. <http://doi.org/10.1111/j.1469-7998.2009.00646.x>
- 955 Kardos, M., Åkesson, M., Fountain, T., Flagstad, Ø., Liberg, O., Olason, P., ... Ellegren, H.
956 (2018). Genomic consequences of intensive inbreeding in an isolated wolf population.
957 *Nature Ecology & Evolution*, 2(1), 124–131. <http://doi.org/10.1038/s41559-017-0375-4>
- 958 Kardos, M., Luikart, G., & Allendorf, F. W. (2015). Measuring individual inbreeding in the
959 age of genomics: marker-based measures are better than pedigrees. *Heredity*, 115(1), 63–
960 72. <http://doi.org/10.1038/hdy.2015.17>
- 961 Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in
962 Ecology and Evolution*, 17(5), 230–241. [http://doi.org/10.1016/S0169-5347\(02\)02489-8](http://doi.org/10.1016/S0169-5347(02)02489-8)
- 963 Knief, U., Kempnaers, B., & Forstmeier, W. (2016). Meiotic recombination shapes precision
964 of pedigree- and marker-based estimates of inbreeding. *Heredity*, (August), 1–10.
965 <http://doi.org/10.1038/hdy.2016.95>
- 966 Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits*.
- 967 Marshall, H. H., Vitikainen, E. I. K., Mwanguhya, F., Businge, R., Kyabulima, S., Hares, M.
968 C., ... Cant, M. A. (2017). Lifetime fitness consequences of early-life ecological
969 hardship in a wild mammal population. *Ecology and Evolution*, 7(6), 1712–1724.
970 <http://doi.org/10.1002/ece3.2747>
- 971 Meagher, S., Penn, D. J., & Potts, W. K. (2000). Male-male competition magnifies inbreeding
972 depression in wild house mice. *Proceedings of the National Academy of Sciences of the
973 United States of America*, 97, 3324–3329. <http://doi.org/10.1073/pnas.97.7.3324>
- 974 Miller, J. M., Malenfant, R. M., David, P., Davis, C. S., Poissant, J., Hogg, J. T., ... Coltman,
975 D. W. (2014). Estimating genome-wide heterozygosity: effects of demographic history
976 and marker type. *Heredity*, 112(3), 240–247. <http://doi.org/10.1038/hdy.2013.99>

- 977 Mitchell, J., Vitikainen, E. I. K., Wells, D. A., Cant, M. A., & Nichols, H. J. (2017).
978 Heterozygosity but not inbreeding coefficient predicts parasite burdens in the banded
979 mongoose. *Journal of Zoology*, 302(1), 32–39. <http://doi.org/10.1111/jzo.12424>
- 980 Morrissey, M. (2014). pedantics: Functions to facilitate power and sensitivity analyses for
981 genetic studies of natural populations.
- 982 Nakagawa, S., & Schielzeth, H. (2013). A general and simple method for obtaining R² from
983 generalized linear mixed-effects models. *Methods in Ecology and Evolution*, 4(2), 133–
984 142. <http://doi.org/10.1111/j.2041-210x.2012.00261.x>
- 985 Nichols, H. J. (2017). The causes and consequences of inbreeding avoidance and tolerance in
986 cooperatively breeding vertebrates. *Journal of Zoology*. <http://doi.org/10.1111/jzo.12466>
- 987 Nichols, H. J., Amos, W., Cant, M. A., Bell, M. B. V., & Hodge, S. J. (2010). Top males gain
988 high reproductive success by guarding more successful females in a cooperatively
989 breeding mongoose. *Animal Behaviour*, 80(4), 649–657.
990 <http://doi.org/10.1016/j.anbehav.2010.06.025>
- 991 Nichols, H. J., Cant, M. A., Hoffman, J. I., & Sanderson, J. L. (2014). Evidence for frequent
992 incest in a cooperatively breeding mammal. *Biology Letters*, 10(12).
993 <http://doi.org/10.1098/rsbl.2014.0898>
- 994 Nichols, H. J., Cant, M. A., & Sanderson, J. L. (2015). Adjustment of costly extra-group
995 paternity according to inbreeding risk in a cooperative mammal. *Behavioral Ecology*,
996 26(6), 1486–1494. <http://doi.org/10.1093/beheco/arv095>
- 997 Nielsen, J. F., English, S., Goodall-Copestake, W. P., Wang, J., Walling, C. A., Bateman, A.
998 W., ... Pemberton, J. M. (2012). Inbreeding and inbreeding depression of early life traits
999 in a cooperative mammal. *Molecular Ecology*, 21(11), 2788–2804.
1000 <http://doi.org/10.1111/j.1365-294X.2012.05565.x>
- 1001 Nietlisbach, P., Keller, L. F., Camenisch, G., Arcese, P., Reid, J. M., & Postma, E. (2017).
1002 Pedigree-based inbreeding coefficient explains more variation in fitness than
1003 heterozygosity at 160 microsatellites in a wild bird population. *Proceedings of the Royal
1004 Society B: Biological Sciences*, 284(1850).
- 1005 O'Quigley, J., Xu, R., & Stare, J. (2005). Explained randomness in proportional hazards
1006 models. *Statistics in Medicine*, 24(3), 479–489. <http://doi.org/10.1002/sim.1946>
- 1007 Ólafsdóttir, G. Á., & Kristjánsson, T. (2008). Correlated pedigree and molecular estimates of
1008 inbreeding and their ability to detect inbreeding depression in the Icelandic sheepdog, a
1009 recently bottlenecked population of domestic dogs. *Conservation Genetics*, 9(6), 1639–
1010 1641. <http://doi.org/10.1007/s10592-008-9526-0>
- 1011 Pemberton, J. M. (2004). Measuring inbreeding depression in the wild: The old ways are the
1012 best. *Trends in Ecology and Evolution*, 19(12), 613–615.
1013 <http://doi.org/10.1016/j.tree.2004.09.010>
- 1014 Pemberton, J. M. (2008). Wild pedigrees: the way forward. *Proceedings of the Royal Society
1015 B: Biological Sciences*, 275(1635), 613–621. <http://doi.org/10.1098/rspb.2007.1531>
- 1016 Pilakouta, N., Jamieson, S., Moorad, J. A., & Smiseth, P. T. (2015). Parental care buffers
1017 against inbreeding depression in burying beetles. *Proceedings of the National Academy
1018 of Sciences of the United States of America*, 112(26), 8031–5.
1019 <http://doi.org/10.1073/pnas.1500658112>
- 1020 R Core Team. (2014). R: A language and environment for statistical computing. *R Foundation
1021 for Statistical Computing, Vienna, Austria, 2012*.
- 1022 Reed, D. H., Fox, C. W., Enders, L. S., & Kristensen, T. N. (2012). Inbreeding-stress
1023 interactions: Evolutionary and conservation consequences. *Annals of the New York
1024 Academy of Sciences*, 1256(1), 33–48. <http://doi.org/10.1111/j.1749-6632.2012.06548.x>

- 1025 Reid, J. M., Keller, L. F., Marr, A. B., Nietlisbach, P., Sardell, R. J., & Arcese, P. (2014).
1026 Pedigree error due to extra-pair reproduction substantially biases estimates of inbreeding
1027 depression. *Evolution*, *68*(3), 802–815. <http://doi.org/10.1111/evo.12305>
- 1028 Richards, S. A., Whittingham, M. J., & Stephens, P. A. (2011). Model selection and model
1029 averaging in behavioural ecology: The utility of the IT-AIC framework. *Behavioral*
1030 *Ecology and Sociobiology*, *65*(1), 77–89. <http://doi.org/10.1007/s00265-010-1035-8>
- 1031 Sanderson, J. L., Wang, J., Vitikainen, E. I. K., Cant, M. A., & Nichols, H. J. (2015). Banded
1032 mongooses avoid inbreeding when mating with members of the same natal group.
1033 *Molecular Ecology*, *24*(14), 3738–3751. <http://doi.org/10.1111/mec.13253>
- 1034 Skaug, H., Fournier, D., Nielsen, A., & Magnusson, A. (2013). Generalized linear mixed
1035 models using AD model builder. *R Package Version 0.7*.
- 1036 Slate, J., David, P., Dodds, K. G., Veenvliet, B. a, Glass, B. C., Broad, T. E., & McEwan, J.
1037 C. (2004). Understanding the relationship between the inbreeding coefficient and
1038 multilocus heterozygosity: theoretical expectations and empirical data. *Heredity*, *93*(3),
1039 255–265. <http://doi.org/10.1038/sj.hdy.6800485>
- 1040 Stam, P. (1980). The distribution of the fraction of the genome identical by descent in finite
1041 random mating populations. *Genetical Research*, *35*(2), 131.
1042 <http://doi.org/10.1017/S0016672300014002>
- 1043 Stoffel, M. A., Esser, M., Kardos, M., Humble, E., Nichols, H., David, P., ... Poisot, T.
1044 (2016). inbreedR: an R package for the analysis of inbreeding based on genetic markers.
1045 *Methods in Ecology and Evolution*, *7*(11), 1331–1339. [http://doi.org/10.1111/2041-](http://doi.org/10.1111/2041-210X.12588)
1046 [210X.12588](http://doi.org/10.1111/2041-210X.12588)
- 1047 Symonds, M. R. E., & Moussalli, A. (2011). A brief guide to model selection, multimodel
1048 inference and model averaging in behavioural ecology using Akaike's information
1049 criterion. *Behavioral Ecology and Sociobiology*, *65*(1), 13–21.
1050 <http://doi.org/10.1007/s00265-010-1037-6>
- 1051 Szulkin, M., Bierne, N., & David, P. (2010). Heterozygosity-fitness correlations: a time for
1052 reappraisal. *Evolution; International Journal of Organic Evolution*, *64*(5), 1202–17.
1053 <http://doi.org/10.1111/j.1558-5646.2010.00966.x>
- 1054 Szulkin, M., Stopher, K. V., Pemberton, J. M., & Reid, J. M. (2013). Inbreeding avoidance,
1055 tolerance, or preference in animals? *Trends in Ecology and Evolution*, *28*(4), 205–211.
1056 <http://doi.org/10.1016/j.tree.2012.10.016>
- 1057 Taylor, H. R., Kardos, M. D., Ramstad, K. M., & Allendorf, F. W. (2015). Valid estimates of
1058 individual inbreeding coefficients from marker-based pedigrees are not feasible in wild
1059 populations with low allelic diversity. *Conservation Genetics*, *16*(4), 901–913.
1060 <http://doi.org/10.1007/s10592-015-0709-1>
- 1061 Taylor, S. S., Sardell, R. J., Reid, J. M., Bucher, T., Taylor, N. G., Arcese, P., & Keller, L. F.
1062 (2010). Inbreeding coefficient and heterozygosity-fitness correlations in unhatched and
1063 hatched song sparrow nestmates. *Molecular Ecology*, *19*(20), 4454–4461.
1064 <http://doi.org/10.1111/j.1365-294X.2010.04824.x>
- 1065 Therneau, T., & Grambsch, P. (2000). *Modeling Survival Data: Extending the Cox Model*.
- 1066 Thompson, F. J., Marshall, H. H., Sanderson, J. L., Vitikainen, I. K., Nichols, H. J., Gilchrist,
1067 J. S., ... Thompson, F. J. (2016). Reproductive competition triggers mass eviction in
1068 cooperative banded mongooses. *Proceedings of Royal Society B*, *283*(1826).
- 1069 Thünken, T., Bakker, T. C. M., Baldauf, S. A., & Kullmann, H. (2007). Active Inbreeding in a
1070 Cichlid Fish and Its Adaptive Significance. *Current Biology*, *17*(3), 225–229.
1071 <http://doi.org/10.1016/j.cub.2006.11.053>
- 1072 Wang, J. (2010). Do marker-based paternity assignments favour heterozygous and unrelated

- 1073 males? *Molecular Ecology*, 19(9), 1898–1913. <http://doi.org/10.1111/j.1365->
1074 294X.2010.04601.x
- 1075 Wang, J. (2016). Pedigrees or markers: Which are better in estimating relatedness and
1076 inbreeding coefficient? *Theoretical Population Biology*, 107, 4–13.
1077 <http://doi.org/10.1016/j.tpb.2015.08.006>
- 1078 Weir, B. S., Avery, P. J., & Hill, W. G. (1980). Effect of mating structure on variation in
1079 inbreeding. *Theoretical Population Biology*, 18(3), 396–429.
1080 [http://doi.org/10.1016/0040-5809\(80\)90061-1](http://doi.org/10.1016/0040-5809(80)90061-1)
- 1081 Zuur, A. F. ., Ieno, E. N. ., & Saveliev, A. (2009). *Mixed Effects Models and Extensions in*
1082 *Ecology with R*. (R. M. Gail, K. Krickeberg, S. JM, T. A, & W. Wong, Eds.). New York,
1083 NY: Spring Science and Bussiness Media.
1084

For Review Only

1085 **Data Accessibility**

1086 Microsatellite genotypes, pedigree inbreeding coefficients, and lifetime and annual data
1087 records are available via Dryad doi:10.5061/dryad.bq868sh. All of the computer code used to
1088 analyse the data are provided as R script files.

1089

1090 **Author Contributions**

1091 J.I.H. and H.J.N. designed the research; D.A.W. genotyped individuals and assigned
1092 parentage and sibships; D.A.W conducted data analyses with assistance from J.I.H.; J.I.H. and
1093 D.A.W. wrote the manuscript with comments from H.J.N.; field data were collected by H.J.N
1094 and M.A.C. All of the authors read and commented upon the final manuscript.

1095

For Review Only

1096 **Tables**

1097

1098 **Table 1.** Alternative models of survival to nutritional independence ranked in order of their
 1099 AIC_c support. See the Materials and Methods section for further details.

Model	Structure	k	logLikelihood	AIC _c	ΔAIC _c	AIC _c -weight
M5	Rain + escorting	5	-271.954	554.033	0.000	0.348
M7	Rain + escorting + sMLH	6	-271.944	556.061	2.029	0.126
M6	Rain + escorting + f_{ped}	6	-271.953	556.081	2.048	0.125
M1	Rain	4	-274.286	556.655	2.623	0.094
M15	Rain * sMLH + escorting	7	-271.866	557.965	3.932	0.049
M11	Rain * f_{ped} + escorting	7	-271.917	558.066	4.034	0.046
M8	Rain + escorting + f_{ped} + residual sMLH	7	-271.939	558.110	4.078	0.045
M3	Rain + sMLH	5	-274.263	558.651	4.618	0.035
M2	Rain + f_{ped}	5	-274.282	558.688	4.655	0.034
M16	Rain * residual sMLH + escorting + f_{ped}	8	-271.811	559.923	5.890	0.018
M12	Rain * f_{ped} + escorting + residual sMLH	8	-271.902	560.103	6.071	0.017
M13	Rain * sMLH	6	-274.182	560.539	6.506	0.013
M9	Rain * f_{ped}	6	-274.203	560.580	6.547	0.013
M4	Rain + f_{ped} + residual sMLH	6	-274.248	560.669	6.637	0.013
M18	Rain * (f_{ped} + residual sMLH) + escorting	9	-271.781	561.937	7.905	0.007
M19	(Intercept only)	3	-278.019	562.087	8.054	0.006
M14	Rain * residual sMLH + f_{ped}	7	-274.091	562.415	8.382	0.005
M10	Rain * f_{ped} + residual sMLH	7	-274.168	562.568	8.536	0.005
M17	Rain * (f_{ped} + residual sMLH)	8	-274.022	564.345	10.312	0.002

1100

1101 **Table 2.** Alternative models of survival beyond nutritional independence ranked in order of
 1102 their AIC_c support. See the Materials and Methods section for further details.

1103

1104

Model	Structure	k	LogLikelihood	AIC_c	ΔAIC_c	AIC_c -weight
M7	Sex + rain + sMLH	8.5	-1645.576	3297.209	0.000	0.261
M11	Sex + rain * sMLH	9.4	-1644.911	3297.916	0.707	0.183
M1	Sex	6.9	-1647.964	3297.938	0.728	0.181
M3	Sex + sMLH	8.1	-1647.174	3298.376	1.167	0.145
M5	Sex + rain	6.3	-1647.560	3299.149	1.939	0.099
M8	Sex + rain + f_{ped} + residual sMLH	7.9	-1646.837	3301.768	4.559	0.027
M2	Sex + f_{ped}	6.6	-1649.023	3302.074	4.865	0.023
M4	Sex + f_{ped} + residual sMLH	7.8	-1648.015	3302.086	4.876	0.023
M6	Sex + rain + f_{ped}	6.6	-1648.164	3302.385	5.176	0.020
M12	Sex + rain * residual sMLH + f_{ped}	8.6	-1646.418	3302.979	5.769	0.015
M10	Sex + rain * f_{ped} + residual sMLH	9.0	-1646.708	3303.559	6.350	0.011
M9	Sex + rain * f_{ped}	7.7	-1648.083	3304.261	7.052	0.008
M13	Sex + rain * (f_{ped} + residual sMLH)	9.7	-1646.283	3304.765	7.555	0.006
M14	(Intercept only)	4.9	-1650.698	3322.777	25.568	0.000

1105

1106 **Table 3.** Alternative models of yearling body mass ranked in order of their AIC_c support. See
 1107 the Materials and Methods section for further details. Only models with AIC_c-weights greater
 1108 than 0.01 are shown.
 1109

Model	Structure	k	logLikelihood	AIC _c	ΔAIC _c	AIC _c -weight
M28	Sex + f_{ped}	6	-930.982	1874.551	0.000	0.325
M32	Sex + rain + f_{ped}	7	-930.896	1876.581	2.029	0.118
M36	Sex + index + f_{ped}	7	-930.935	1876.659	2.107	0.113
M30	Sex + f_{ped} + residual sMLH	7	-930.955	1876.699	2.147	0.111
M43	Sex + rain * f_{ped}	8	-930.509	1878.039	3.488	0.057
M34	Sex + rain + f_{ped} + residual sMLH	8	-930.849	1878.719	4.168	0.040
M40	Sex + rain + index + f_{ped}	8	-930.849	1878.719	4.168	0.040
M38	Sex + index + f_{ped} + residual sMLH	8	-930.910	1878.841	4.290	0.038
M44	Sex + rain * f_{ped} + residual sMLH	9	-930.436	1880.158	5.606	0.020
M45	Sex + escorting + rain * f_{ped}	9	-930.448	1880.182	5.630	0.019
M48	Sex + rain * residual sMLH + f_{ped}	9	-930.644	1880.574	6.022	0.016
M42	Sex + rain + index + f_{ped} + residual sMLH	9	-930.804	1880.894	6.342	0.014
M27	Sex	5	-935.417	1881.251	6.699	0.011
M2	f_{ped}	5	-935.495	1881.407	6.855	0.011

1110

1111 **Table 4.** Alternative models of annual reproductive success in (a) females, and (b) males,
 1112 ranked in order of their AIC_c support. The models of female annual reproductive success
 1113 which included inbreeding–stress interactions failed to converge and so were omitted. See the
 1114 Materials and Methods section for further details.

1115

1116 (a)

Model	Structure	k	logLikelihood	AIC _c	ΔAIC _c	AIC _c -weight
M4	Age + age ² + f_{ped}	8	-329.679	675.981	0.000	0.286
M1	Age + age ²	7	-330.848	676.179	0.197	0.259
M5	Age + age ² + rain + f_{ped}	9	-329.642	678.067	2.085	0.101
M8	Age + age ² + f_{ped} + residual sMLH	9	-329.652	678.087	2.105	0.100
M3	Age + age ² + sMLH	8	-330.790	678.203	2.222	0.094
M2	Age + age ² + rain	8	-330.808	678.239	2.258	0.092
M7	Age + age ² + rain + f_{ped} + residual sMLH	10	-329.625	680.211	4.229	0.034
M6	Age + age ² + rain + sMLH	9	-330.733	680.249	4.267	0.034
M14	(Intercept only)	5	-369.474	749.204	73.223	0.000

1117

1118 (b)

Model	Structure	k	logLikelihood	AIC _c	ΔAIC _c	AIC _c -weight
M8	Age + age ² + f_{ped} + residual sMLH	9	-300.139	618.801	0.000	0.494
M7	Age + age ² + rain + f_{ped} + residual sMLH	10	-300.133	620.907	2.106	0.172
M12	Age + age ² + rain * residual sMLH + f_{ped}	11	-299.697	622.166	3.365	0.092
M10	Age + age ² + rain * f_{ped} + residual sMLH	11	-300.051	622.874	4.073	0.065
M3	Age + age ² + sMLH	8	-303.333	623.083	4.282	0.058
M4	Age + age ² + f_{ped}	8	-303.792	624.001	5.200	0.037
M13	Age + age ² + rain * (f_{ped} + residual sMLH)	12	-299.663	624.241	5.440	0.033
M6	Age + age ² + rain + sMLH	9	-303.332	625.187	6.386	0.020
M5	Age + age ² + rain + f_{ped}	9	-303.779	626.081	7.280	0.013
M11	Age + age ² + rain * smlh	10	-302.895	626.431	7.630	0.011
M9	Age + age ² + rain * f_{ped}	10	-303.725	628.091	9.290	0.005
M1	Age + age ²	7	-309.393	633.110	14.308	0.000
M2	Age + age ² + rain	8	-309.390	635.197	16.396	0.000
M14	(Intercept only)	5	-343.651	697.474	78.673	0.000

1119

1120 **Table 5.** Statistical significance and variance explained by inbreeding terms in models of five
 1121 fitness traits. The significance of f_{ped} and sMLH was derived by comparing models containing
 1122 these terms with equivalent 'null models' containing only the relevant non-inbreeding terms,
 1123 while p -values for residual sMLH were obtained through the comparison of models
 1124 containing f_{ped} + residual sMLH with equivalent models containing only f_{ped} . For each trait,
 1125 the models that we constructed are listed in the first column of the table, with the null model
 1126 shown first. Conditional R^2_{glmm} was calculated following Nakagawa & Schielzeth (2013) and
 1127 Cox and Snells's pseudo R^2 was calculated using the number of uncensored observations
 1128 rather than the total number of observations as recommended by O'Quigley et al. (2005). As
 1129 R^2 values cannot be calculated for zero-inflated negative binomial GLMMs, log likelihood
 1130 values are presented as a measure of the fit of models of annual male reproductive success.
 1131

a) Survival to nutritional independence

Binomial GLMM, $n = 489$

Structure	Likelihood ratio	p -value	Conditional R^2_{glmm}
Rain + escorting			0.4701
Rain + escorting + f_{ped}	0.0017	0.9671	0.4702
Rain + escorting + sMLH	0.0213	0.8839	0.4703

b) Survival beyond nutritional independence

Cox proportional hazard model, $n = 428$

Structure	Likelihood ratio	p -value	Cox and Snell's pseudo R^2
Sex			0.0817
Sex + f_{ped}	2.1178	0.1456	0.0755
Sex + sMLH	1.5803	0.2087	0.0863

c) Yearling body mass

Gaussian GLMM, $n = 150$

Structure	Likelihood ratio	p -value	Conditional R^2_{glmm}
Sex			0.5734
Sex + f_{ped}	8.87	0.0029	0.6221
Sex + sMLH	0.674	0.4117	0.5766

d) Female annual reproductive success

Zero-inflated, negative binomial GLMM, $n = 240$

Structure	Likelihood ratio	p -value	Log Likelihood
Age + age ²			-330.848
Age + age ² + f_{ped}	2.338	0.1263	-329.679
Age + age ² + sMLH	0.116	0.7334	-330.790

e) Male annual reproductive success

Zero-inflated, negative binomial GLMM, $n = 354$

Structure	Likelihood ratio	p -value	Log Likelihood
Age + age ²			-309.393
Age + age ² + f_{ped}	11.202	0.0008	-303.792
Age + age ² + sMLH	12.12	0.0005	-303.333
Age + age ² + f_{ped} + residual sMLH	7.306	0.0069	-300.139

1132

1133

For Review Only

1134 **Figure legends**

1135

1136 **Figure 1.** The relationship between the pedigree-based inbreeding coefficient, f_{ped} and sMLH
1137 for 777 banded mongoose individuals with all four grandparents assigned ($R = 0.34$, $p <$
1138 0.001). Scatter on the y-axis for a given f_{ped} value represents variation in microsatellite
1139 heterozygosity among individuals with the same pedigree inbreeding coefficient. Marginal
1140 histograms show the distributions of f_{ped} (top) and sMLH (right axis).

1141

1142 **Figure 2.** The relationship between f_{ped} and yearling body mass. The trend line shows the
1143 expected body mass of a female yearling and the shaded region shows the 95% confidence
1144 interval.

1145

1146 **Figure 3.** The relationship between annual male reproductive success and a) f_{ped} , and b)
1147 residual sMLH derived from a single model (M8 in table 4b) where both inbreeding measures
1148 are fitted together. The trend line shows expected values based on average age and the shaded
1149 region shows associated 95% confidence intervals. Data points in plot a) were given a small
1150 amount of jitter to avoid over plotting.

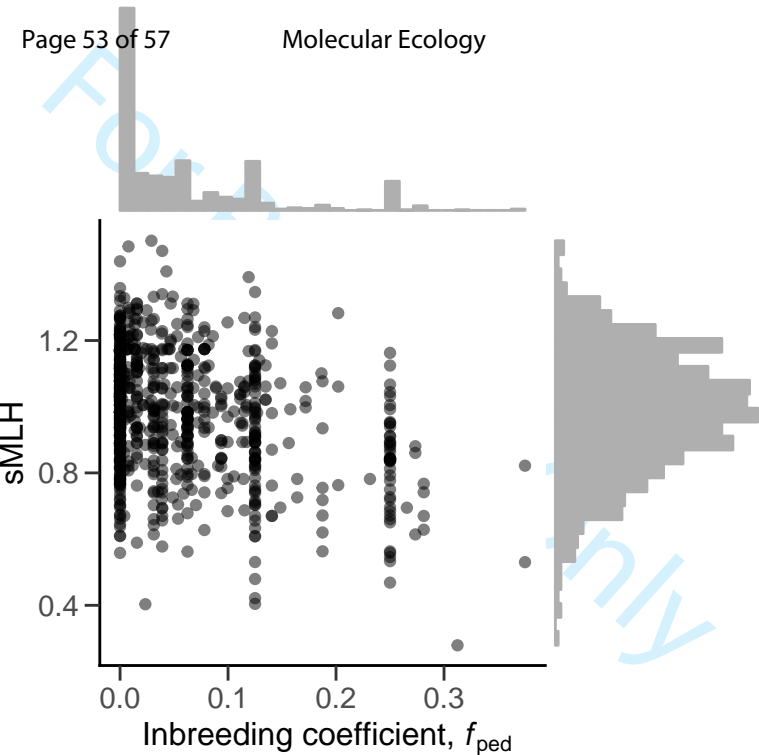
1151

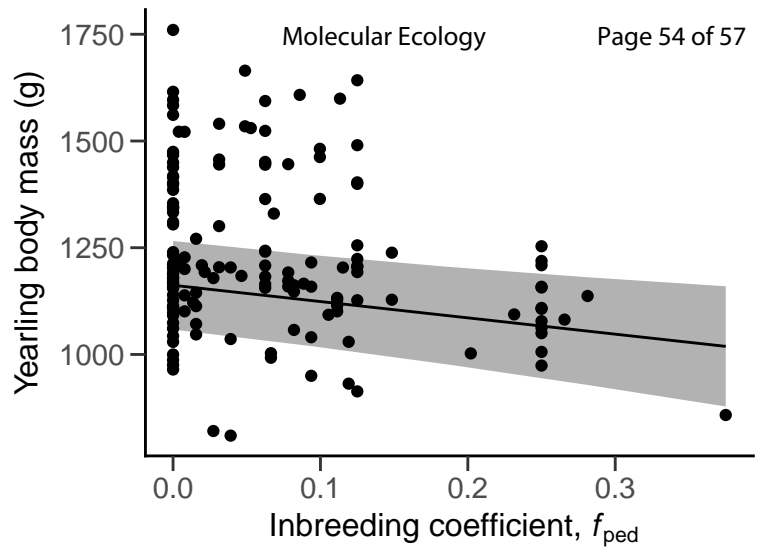
1152 **Figure 4.** Estimated regression coefficients of the three inbreeding terms in models of five
1153 different fitness traits, showing point estimates and associated 95% confidence intervals.
1154 Each panel shows three different models—one containing f_{ped} (shown in black), one containing
1155 sMLH (shown in dark orange) and one containing f_{ped} + residual sMLH (shown in light
1156 turquoise) as described in the Results section. In addition to these inbreeding terms, all of the
1157 models contained other fixed effects but these are not shown for ease of interpretation. The
1158 larger confidence intervals of f_{ped} relative to sMLH result from its smaller range (Figure 1).

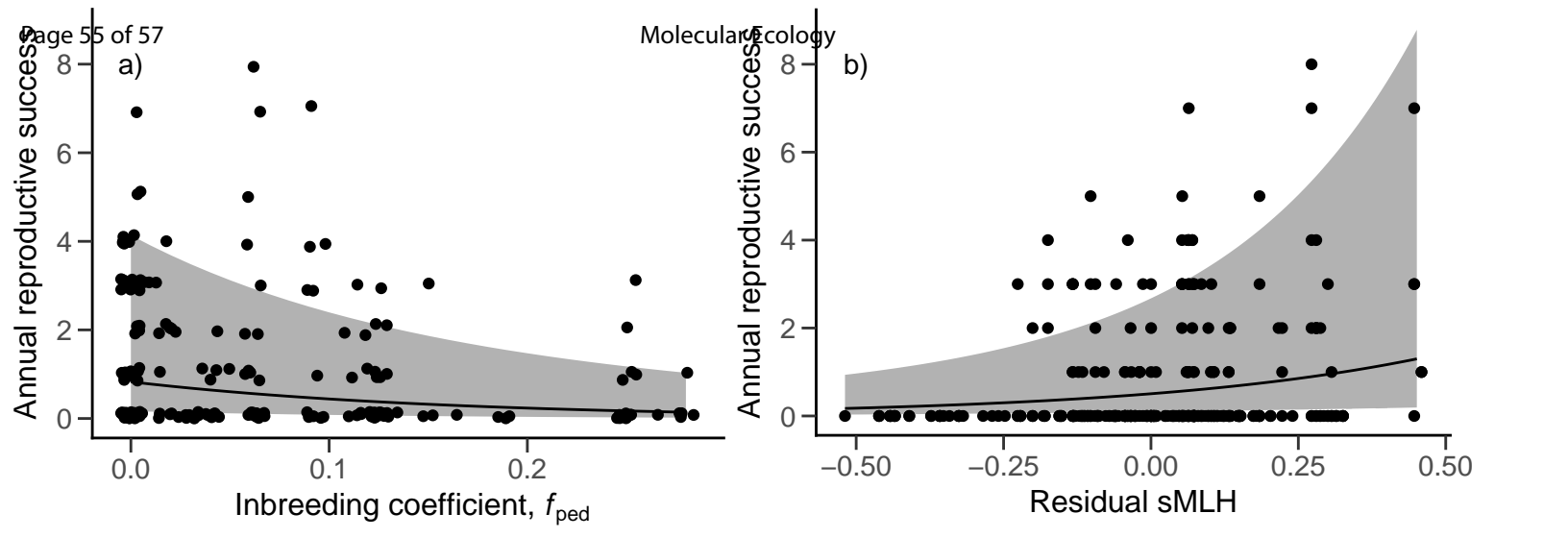
1159

1160 **Figure 5.** The relationship between AIC_c of models of annual male reproductive success and
1161 the number of microsatellites used to calculate standardised multilocus heterozygosity. Open
1162 points represent models with the structure: age + age² + sMLH; closed points represent
1163 models with the structure: age + age² + f_{ped} + residual sMLH. The horizontal line represents a
1164 model with the structure: age + age² + f_{ped} . We selected n different microsatellite loci at
1165 random and calculated heterozygosity as sMLH 100 times for each value of n . Points
1166 represent mean values and the shaded regions indicate $\pm 1\text{sd}$.

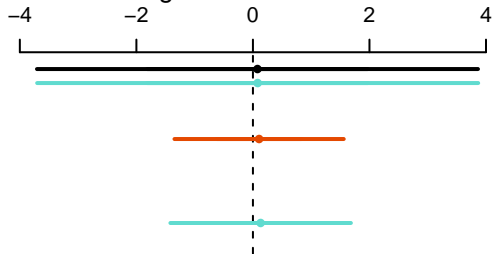
1167





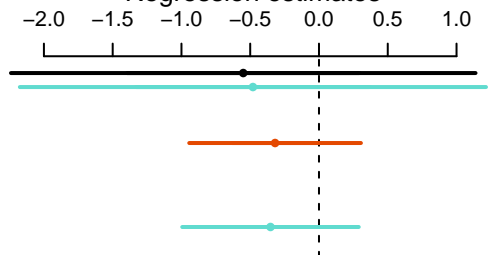


Regression estimates



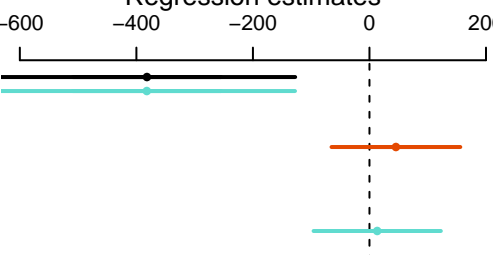
b) Survival beyond nutritional independence

Regression estimates



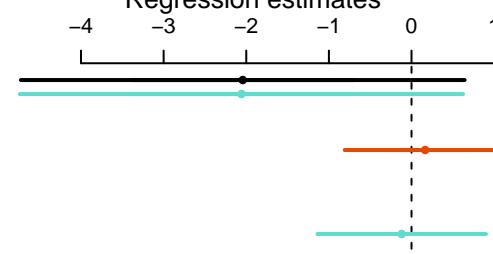
c) Yearling body mass

Regression estimates



d) Female annual reproductive success

Regression estimates



e) Male annual reproductive success

Regression estimates

