Adv Physiol Educ 49: 1142–1148, 2025.
First published October 18, 2025; doi:10.1152/advan.00085.2025

american physiological society

ADVANCES IN PHYSIOLOGY EDUCATION

SOURCEBOOK OF LABORATORY ACTIVITIES IN PHYSIOLOGY

Drosophila melanogaster as a physiologically relevant invertebrate teaching model system of complex neurological disease

James L. Rouse,[1,2] Laura F. Corns,[3] Aidan Seeley,[4] and Nicholas S. Freestone[5]

[1]*School of Biology, University of Leeds, Leeds, United Kingdom;* [2]*Applied Insect Science, Ripon, United Kingdom;* [3]*School of Biosciences, University of Sheffield, Sheffield, United Kingdom;* [4]*Swansea University Medical School, Swansea University, Swansea, United Kingdom; and* [5]*School of Life Sciences, Pharmacy and Chemistry, Kingston University, London, United Kingdom*

Abstract

In response to financial, logistical, and ethical pressures, universities are exploring innovative methods for teaching physiology practicals with animal models. This study presents a laboratory activity employing *Drosophila melanogaster* as a model for neurological disease, leveraging its historical utility in genetic and physiological research. As invertebrates, *D. melanogaster* are not subject to the Animal (Scientific Procedures) Act 1986 in the United Kingdom, making them suitable for large-class teaching. The activity aims to enhance students' molecular skills and understanding of genotype-phenotype linkages through hands-on experiments. Students conduct DNA extraction, PCR, and restriction digestion, followed by behavioral assays to assess motor function. Results demonstrate consistent molecular outcomes and significant differences in climbing ability between wild-type and mutant flies, mirroring multiple human neurological disease symptoms. The practical encourages inquiry-based learning, allowing students to design multistage experiments and analyze complex data. This comprehensive approach not only reinforces theoretical knowledge but also provides valuable insights into human disease mechanisms with invertebrate models. The methodology can be adapted for various educational levels and expanded to include more advanced techniques such as qPCR, fostering a deeper understanding of molecular biology and neurophysiology.

NEW & NOTEWORTHY There are ethical revisions around the use of animal models in research and teaching. However, there is still a need to train students in physiological techniques to promote skill development and engagement in research. This article provides an ethically accessible, inquiry-based practical using *Drosophila melanogaster* to model neurological disease. The activity reinforces core physiological and molecular skills while fostering analytical thinking and engagement with human disease mechanisms.

Drosophila melanogaster; partial replacement; 3Rs; undergraduate experiments

INTRODUCTION

Undergraduate education in biology has moved away from using animal models in teaching. In the United Kingdom and Europe, financial, logistical, and ethical issues have been raised about the use of animal models in nonresearch contexts and in the spirit of practicing the three Rs (replacement, reduction, and refinement) (1). In the United States, legislation around these issues is less pressing, though there are still financial and logistical arguments for introducing nonmammalian models into undergraduate teaching, and institutions may still voluntarily implement ethical processes for invertebrate research. Overall, changing opinion requires educational institutions to think of alternative ways to present teaching to allow the development of in vivo skills that are important to the development of students' learning and are still seen as core skills by potential employers (2, 3).

In addition to the need to move away from animal models in teaching, there is also a growing emphasis on student-centered learning. This approach fosters critical thinking by encouraging students to connect apparently disparate skills to answer an overarching question (4, 5) and promotes a learning environment that mirrors the complexity of research work. With the potential of more complex practical classes required to deliver these critical thinking skills, and the movement away from higher-order animal use in educational scenarios, the low cost of acquisition and maintenance of invertebrate models becomes all the more important to future educational endeavors (6).

Here, we present a laboratory activity using *Drosophila melanogaster* as a model for neurological diseases defined by mutations in a sodium-potassium pump affecting neuromotor function. The historical utility of *D. melanogaster* as a model organism allows both physiological and genetic aspects of neurological disease to be evaluated (7). This can

Correspondence: J. L. Rouse (jrouse@live.co.uk).
Submitted 4 April 2025 / Revised 3 July 2025 / Accepted 12 October 2025

be used to promote student learning in designing multistage experiments that connect multiple different knowledge areas as part of a larger question in physiological teaching. As invertebrates, *D. melanogaster* are not covered by the Animal (Scientific Procedures) Act 1986 in the United Kingdom or the Animal Welfare Act Regulations or the Public Health Service Policy on Humane Care and Use of Laboratory Animal in the United States. They can therefore be utilized for large-class teaching while addressing logistical, financial, and ethical concerns associated with the use of mammalian models in teaching. As an added benefit, hands-on work with *Drosophila* allows students to develop skill-based learning with an extensively used model organism.

Objectives and Overview

The laboratory activity described provides students with the opportunity to gain relevant molecular skills and link these to phenotypic affects in a disease model organism. The genotypic/phenotypic change can be adapted as long as the molecular visualization is easy to link with the phenotypic outcome and therefore provides students with a relatively simple connection between genotype and phenotype.

The objectives of the activities described in this practical exercise are *1*) to conduct common molecular skills, *2*) to conduct phenotypic experiments relevant to neurophysiology, *3*) to analyze complex and potentially messy data, and *4*) to understand how to design multistage experiments to explore genotypic effects on physiological function.

Background

The fruit fly (*Drosophila melanogaster*) can be used as a model to investigate the mechanisms underlying a variety of phenotypes. The fly has been used as a model organism to understand human genetic diseases for ~100 years and was one of the first organisms to be sequenced back in 2000. Around 60% of human genes have an ortholog in the fly, with that percentage increasing to 75% when discussing human genes involved in disease (8).

Mutations affecting *ATPalpha* in humans have been implicated in multiple neurological diseases, such as epilepsy (9), familial hemiplegic migraine (10), Charcot–Marie–Tooth disease (11, 12), and rapid-onset dystonia-parkinsonism (13). In *Drosophila*, loss-of-function mutations in the gene allow us to explore functionally whether this gene is responsible for other, disease-related, phenotypic changes. The mutant strain used in this practical class was generated by chemical treatment with ethyl methanesulfonate that caused a heterozygous missense mutation in the *ATPalpha* gene located on the 3rd chromosome (10). This mutation is homozygous lethal but is considered to be hypomorphic, i.e., causing partial loss of gene function, as the mutation does not affect all functions of the ATPalpha protein. As the mutation is a simple G to A substitution, this creates a restriction enzyme site that can be used to digest the eventual PCR product. The mutant can therefore be compared to wild type via simple molecular methods to effectively show students the difference between a wild-type and a genetically modified fly.

Patients suffering from neurological diseases often show multiple symptoms such as loss of memory and movement disorders. These phenotypes are easily tested in the fly and are an important resource for in vivo studies. Mutation of $ATPalpha^{CJ10}$ in *Drosophila* causes a decrease in climbing ability (equivalent to motor skill impairment in humans) and deficits in learning and memory, phenotypes also observed in neurological disease (10). Within this practical, movement will be assessed via a simple behavioral experiment, the negative geotaxis assay. This takes advantage of flies' innate behavior to climb in opposition to gravity after being knocked to the bottom of a vertical surface. The speed at which flies subsequently climb the surface can be measured and used to assess locomotion.

This practical will provide methodology outlining handling a commonly used model organism, simple behavioral experiments, and powerful molecular biology tools (DNA extraction, PCR). As the practical can be run over multiple sessions, students can be given the opportunity to discuss development of experimental design and analysis and explore how separate techniques can be combined into one practical sequence. If desired, the general outline and timing of the practical can be adapted to other readily available *Drosophila* mutants provided the molecular aspects of the practical are known.

Learning Objectives

After completing the laboratory activity, students should be able to

1) Explain the use of invertebrate models in the study of human disease.
2) Describe phenotype/genotype linkage and PCR as a common molecular biology technique.
3) Design multistage experiments to explore genotypic effects on physiological function.
4) Analyze self-collected, potentially "messy" data.

Activity Level

The laboratory activity described below was used with final-year undergraduate biology students as part of COVID catch-up practicals, designed as skills-based learning to develop students' practical abilities in a short period of time after the COVID pandemic. In a more traditional setting, the classes would be suitable for second-year undergraduates using mutants with previously known phenotypes to ensure comprehension of the molecular portion of the practical.

Work using this method has also been employed with final-year undergraduates and Master's students at a project level as a way to generate meaningful data, for students to design their own research, and for students to understand the linkage between discrete techniques while learning new skills.

Prerequisite Knowledge

Students participating in this lesson should have had exposure to standard laboratory micropipettes and solution handling, microscopes, and basic statistics and should have a basic understanding of genetics. Classroom

instructors should understand the theory of PCR and should have experience with invertebrate disease models. Previous work with *Drosophila* is beneficial but is not required.

Students should have knowledge of animal models in research and the principles of replacement, reduction, and refinement (the 3Rs). Students who prefer not to work with living organisms can share datasets from the class. Molecular skills can be learned with previously extracted DNA.

Time Required

The molecular aspects of the work detailed below take a maximum of 4 h but can be paused and samples can be frozen to be picked up at a later date. This feature greatly enhances the flexibility of the learning opportunities afforded by these techniques. Behavioral experiments take up to 2 h for negative geotaxis and classroom analysis.

METHODS

Equipment and Supplies

Molecular assay.

Deionized H_2O

Lysis solution (25 mM NaOH, 0.2 mM Na_2EDTA; from Ref. 14)

Neutralizing solution (40 mL Tris-HCI; from Ref. 14)

Ice

Wild-type flies (designated WT)

ATPalpha mutant flies (designated ATP)

PCR reaction mix: usually a combination of 10× PCR buffer, $MgCl_2$, dNTPs, Taq polymerase

Primer mix (forward: tgcctaccaaaatgcctaaataaa; reverse: cagattcctgccatatcactgg)

10× CutSmart buffer

Mlyl restriction enzyme

TBA buffer

Agarose gel equipment: agarose, GelRed, gel tray, gel comb, gel viewer, loading dye, 100-bp ladder

PCR machine

NanoDrop/spectrophotometer

Micropipettors

Behavioral assay.

Dissecting microscope

Small paint brushes

Glass milk bottle: anything that will keep cold when in ice

Glass/plastic petri dish

Funnel

2× Polystyrene *Drosophila* vials: recommend Flystuff narrow *Drosophila* vials from SLS (FLY1190)

Stopwatch/phone timer

Pen

Drosophila

Wild-type drosophila can be obtained from many providers. The most common bought wild-type lines can be found at Bloomington *Drosophila* Stock Center (Indiana University Bloomington) and Vienna *Drosophila* Resource Center.

ATPalpha^{CJ10} mutant flies can be obtained from Amanda Bretman's laboratory at the University of Leeds.

As invertebrates, *Drosophila* are not governed by the Animal (Scientific Procedures) Act, 1986; however, adopters of this activity are responsible for obtaining permission for human or animal research from their home institution. Similar legislative frameworks are likely to exist in many other countries. For a summary of the Guiding Principles for Research Involving Animals and Human Beings, please see https://www.physiology.org/mm/Publications/Ethical-Policies/Animal-and-Human-Research.

Instructions

Teaching and laboratory timing.

Completion of this laboratory exercise can be performed in four sessions, each between 1.5 and 3 h long (Table 1).

The first session is dedicated to discussion of the session, introduction of disease models, and discussion of experimental design. Practically, this first session also involves DNA extraction, PCR, and restriction digestion.

The second session introduces working with the *Drosophila* model and negative geotaxis data collection. Also covered is how to anesthetize flies with ice and manipulate them without injury to the fly. Multiple phenotypes of flies are introduced to the students, with each individual phenotype being represented in the mutant model. This encourages the students to ask questions around why multiple phenotypes are contained in the mutant model and promotes discussion of how to control for genotype when designing experiments (balancing of genetic crosses). Negative geotaxis is also demonstrated to the students.

The third session allows students to make gels and run their PCR product. The run time of gel electrophoresis allows for class discussion of the theories covered so far and student analysis of the previously collected negative geotaxis data. To expedite the class, this session can be accelerated by having premade gels ready for the students to use.

The fourth session allows for discussion of results. A verbal summary with appropriate slides is presented to strengthen the link between human physiological disease and the power of invertebrate versus vertebrate models for understanding these diseases. Other disease models can also be discussed that utilize methods similar to this practical to

Table 1. *Representative timings and scheduling of the laboratory sessions associated with this model*

Practical Day	Session Timings	Practical
1	10:00–1:00	General introduction DNA extraction PCR Restriction digest (can be performed at the start of the next session)
	2:00–5:00	Intro to "fly pushing" Negative geotaxis (collect data)
2	10:00–1:00	Make gels Run PCR product
	2:00–3:30	Basic statistics Discussion/quiz and sum-up

show the transferability of the skills and ideas learned (15). At the same time, students are led through a series of questions designed to establish learning and help students think about the experiment as a whole (the Padlet platform was used for this). Some questions focus on the correct statistical tests to use to compare control and mutant movement and other potential physiological measures to include in the sessions (learning and memory measurements, possible dissection, etc.) to increase understanding of the disease phenotype. This is also an opportunity to collect feedback on the session.

To ensure all that students have the opportunity to move through the protocol, each student is provided with an entire set of laboratory consumables and pipettes. PCR machines and gel electrophoresis machines are shared. However, to reduce the session time and to promote teamwork and peer learning, this protocol could easily be adapted to allow group working.

Molecular work.

1) Transfer 1 fly of each genotype into 50 μL of Lysis solution (there is no need to crush the fly) and incubate at 95°C for 30 min. Place the samples on ice for 5 min.
2) Add 50 μL of Neutralizing solution and mix. This liquid now contains the DNA required for a PCR reaction.
3) Measure DNA concentration via relevant method (NanoDrop, etc.) and dilute DNA to 25 ng/μL if required.
4) Set up PCR by adding all reagents in Table 2 (× 4 reactions) except extracted DNA into a sterile Eppendorf. This is now the mastermix (specific mastermix makeup and reaction details used in current practical in Table 2).
5) Pipette 23 μL of mastermix into 4 PCR tubes and to each tube add 2 μL of relevant extracted DNA. DNA added should be from wild-type extraction, from ATPalpha extraction, negative control (water), and positive control (instructor-extracted DNA).
6) Set up restriction digest by adding reagents (Table 2) excluding PCR product into 2 clean PCR tubes. To 1 tube add 14 μL of the WT PCR product. In the other tube add 14 μL of the ATPalpha mutant PCR product.
7) Incubate reactions for 18 h at 37°C.
8) Make an agarose gel and run restriction digest product, negative and positive control; 2% agarose gel works well for this product. Gels can be premade, or students can be taken through the process of making their own gels.

Table 2. *Reagent details for molecular assay*

PCR Setup		
PCR reagents	**Volume, μL**	**× 4 Reactions (+ 10%)**
dH_2O	17.35	76.34
10× buffer	2.5	11
$MgCl_2$ (25 mM)	1.6	7.04
dNTPs (10 mM)	0.5	2.2
Primer mix (5 μM each)	0.75	3.3
Taq polymerase	0.3	1
PCR Reaction		
Step	**Temperature**	**Duration**
1	95°C	3 min
2	94°C	30 s
3	57°C	1 min
4	72°C	40 s
5	Go to 2, 34 times	
6	72°C	10 min
7	4°C	Hold
Restriction Digest		
Reagent		**Volume, μL**
dH_2O		2.05
10× CutSmart Buffer		1.9
MlyI restriction enzyme		1.05
PCR product		14
Total		19

Suggested volumes for PCR and restriction digest molecular reactions are shown. The PCR reaction includes +10% overage to account for potential loss of liquid in the pipetting process.

Fly work.

1) Put a glass bottle into ice to chill and fill petri dish with ice. Anesthetize flies by knocking them from a *Drosophila* vial (*Drosophila* vial from SLS) into the chilled bottle via a funnel and tipping onto the chilled petri dish. It is possible that flies' wings will stick to the petri dish if moisture is present. Before tipping the flies onto the dish, the dish should be wiped clean with a dry cloth.
2) Move a single fly from the chilled petri dish into a clean *Drosophila* vial and wait until the fly has recovered (this may take up to 5 min). During this time the *Drosophila* vial containing the fly can be marked in 1-cm increments with a permanent marker.
3) Timer to be set for 3 s. The vial containing a fly is firmly tapped onto a bench top 3 times. To simplify the practical and reduce resources only 1 fly needs to be used per vial. However, multiple flies can be used. At the same time as the last of these taps, the timer needs to be started and the fly followed up the vial.
4) At the end of the 3 s, the distance covered (in cm) by the fly is recorded. This should be repeated 3 times for each fly. The process should be repeated for 5 flies of each genotype, using the same test vials each time.
5) The control flies should progress up the vial after the final tap. For most of these flies, all 3 repeats would be expected to reach the top of the vial by the end of the 3-s period.
6) The test flies are mutant for *ATPalpha*^{CJ10}, a mutation that impairs motor skills in humans and mirrors neurological disease phenotypes. We would expect the flies to react differently from the controls by struggling to move up the vial and display negative geotaxis.
7) An average should be taken of the 3 technical repeats for each fly and entered into a class database. In this way each student should have 5 biological repeats for wild-type and mutant flies. The students may then analyze their own data, or group data, depending on group size.

Troubleshooting

As with many practicals involving the activity of invertebrate models, specific environmental conditions are required

for results to be consistent and predictable. The temperature in the laboratory will ideally be above 20°C to allow the *Drosophila* to move naturally and to ensure that control flies can move quickly against gravity during the negative geotaxis assay.

The molecular biology steps require some confidence with micropipetting, and less confident students may struggle. At each step, pre-prepared solutions can be made so that students might be able to continue to the practical's end if something goes wrong.

Using a class-developed averaged dataset ensures that negative geotaxis results show a significant decrease in the disease model and leads students to think about the importance of biological replication in their own experiments.

Safety Considerations

Basic laboratory safety should be followed at all times; students should wear laboratory coats and protective gloves.

RESULTS

Figure 1 illustrates the kind of class data expected for both negative geotaxis (Fig. 1*A*) and the molecular aspects of the class (Fig. 1*B*). The molecular aspects are expected and should not change. In our experience, when running this practical ~70% of students successfully run through the entire protocol and achieve a gel showing on band for wild-type flies (Fig. 1*B*, *center*) and two or three bands for mutant flies as expected (Fig. 1*B*, *right*). The most often experienced problem with the molecular biology part of the class would be for there to be no bands present at all. If this occurs, the student has most likely set up the PCR reaction incorrectly or punctured the bottom of the gel. Another reason may be incorrect DNA extraction, which is why students are told to check their DNA concentrations on a NanoDrop before entering them into the PCR reaction. An easy way to check whether students have set up a PCR reaction incorrectly is to check for the presence of the ladder (Fig. 1*B, left*). If present, this suggests that students have correctly pipetted into the gel and that their PCR reactions are incorrect.

The negative geotaxis assay is expected to provide consistent results whereby the wild-type flies climb higher than mutant flies (Fig. 1*A*). This can easily be linked to human clinical pathophysiological features of diseases involving movement difficulties, with this being stressed to students in the concluding discussion of the practical. This is especially relevant when students closely monitor the flies, as ATPalpha express a bang-sensitive phenotype, meaning the *ATPalpha* flies show signs of paralysis if treated roughly in their vials. Indeed, if students notice this phenotype there should be discussion around this phenotypic similarity to epilepsy symptoms, of which this mutant is a model. Students should be directed to think about what this may mean in terms of the physiology and clinical symptoms associated with neurological diseases and what mutant flies can tell us about disease phenotypes. This bang sensitivity is one of the reasons mutant flies will climb less distance than control flies in the negative geotaxis assay but will not be triggered outside of the assay by even the most heavy-handed student.

Depending on the class size, the data generated in the negative geotaxis assay may not provide results showing a significant difference between control and *ATPalpha* mutants. If this occurs, this offers opportunities to discuss the importance of biological replication (especially if class numbers are low) and treatment of outliers in behavioral data and to stress that the use of an invertebrate model allows for the ethical accumulation of large amounts of biological information. If the environment in the laboratory is hostile to the flies, i.e., it is cold, then the wild-type flies will not climb in the negative

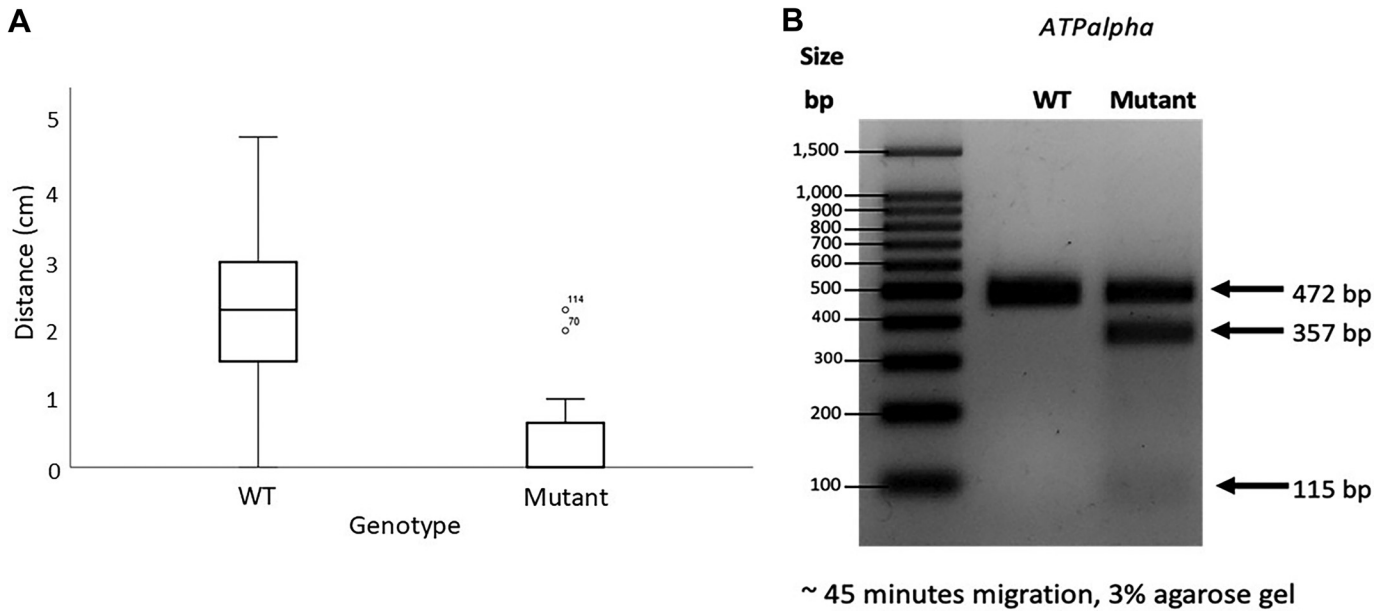

Figure 1. *A* shows the results of a geotaxis assay and how far different variants of the flies can move. This represents replication of data from $n = 67$ students, each with 1 control and 1 mutant fly, so it should be achievable in an undergraduate setting; however, this level of replication is not required for significance. WT, wild type. *B* shows a representative gel with amplification of DNA signals via PCR. Three bands are shown, a WT band as the mutant is heterozygous and 2 bands (257 and 115) representing the PCR product after undergoing enzyme reaction.

geotaxis assay and the data will look very similar to the mutant data below. However, if the laboratory environment is at 20°C or higher this assay is robust and reproducible.

Evaluation of Student Work

Activities described in this model are suitable for second- or third-year undergraduate students with an opportunity to put theory learning into practice regarding the genotype-to-phenotype connection and the value of invertebrate models to investigate human disease. The value of the exercise lies in its comprehensiveness, allowing students to make links between theory taught in prior years and their subsequent practical experience.

Misconceptions

A common misconception before presenting this practical to students is a belief that invertebrate models cannot give useful insights into human (vertebrate) neurological or motor disorders. Effectively linking a direct genotype-phenotype connection allows students to understand the power of invertebrates as a first-pass model to understand the mechanism of human disease. This also allows teaching staff to discuss how this initial replacement of mammalian models can lead to mammalian model refinement in future experimental work.

Another misconception addressed is the fact that an environmental insult cannot be used to test complex physiological mechanisms and their treatments. For example, a "bang" can be easily used in this and other models to switch on a paralyzed state in the affected flies. This then opens up the model to the testing of, for example, antiepileptic drugs (16).

Inquiry Applications

The experiments outlined here lend themselves to a "Methods" level of inquiry as described above, as the practical is designed to expose students to the largest possible number of common molecular methodologies while focusing on a physiological question. This practical can easily be made more student centered via the introduction of multiple easy physiological assays while keeping the core molecular understanding. For example, there are multiple learning and memory tests [courtship conditioning (17), proboscis extension response (18)] that can easily be performed in an undergraduate laboratory that will reveal neural defects in relevant *Drosophila* mutants. There are also multiple measures of movement [DAM tracking (19)] and other behavioral assays (20) that can be easily performed. The basis of the protocol could also be easily adapted for *Drosophila* larvae, where again multiple easy physiological experiments are well documented. For example, the use of larvae as a model for impact of dietary health on development (21) or as a model for precision toxicology (22) has already been described.

The model can also serve as an introduction to further methodology that can used as an open inquiry. There are many mutant *Drosophila* strains that are models of disease that have been underexplored in terms of specific physiological testing, i.e., learning and memory. An expansion of the class to a semester-long "open inquiry" where students pick/design physiological measures of learning and memory and movement and characterize mutations has been used previously at the University of Leeds in the United Kingdom as a third year (final year) capstone project.

Wider Educational Applications

Although here we have presented PCR as a technique to link phenotype and genotype, there are opportunities to develop this protocol to introduce other, more recent, techniques for creating and exploring invertebrate disease models. As the primer information used for the molecular section of the practical is given to students, students could be introduced to BLAST to allow them to explore the PCR product, identifying the product as 472 base pairs long and showing the students where the expected restriction enzyme activity is expected to take place within the mutants. This would have the added benefit of allowing students to engage with basic bioinformatic techniques, potentially further promoting understanding of the invertebrate model.

To introduce new techniques for creating disease models, RNAi disease models could be used to reduce levels of a gene instead of mutating it, leading to the need to teach qPCR in the undergraduate curriculum. However, this would require an increase in the difficulty of the session and the theory taught. This need may be offset by utilizing a whole semester of teaching time to mitigate against the increased complexity. Indeed, multiple suggestions have already been made to allow for qPCR teaching (23), including in *Drosophila* (24).

DATA AVAILABILITY

Data will be made available upon reasonable request.

ACKNOWLEDGMENTS

The authors acknowledge Jennifer Ogbeta for technical assistance in setting up the practical.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.L.R. conceived and designed research; J.L.R. performed experiments; J.L.R. analyzed data; J.L.R. interpreted results of experiments; J.L.R. prepared figures; J.L.R. and A.S. drafted manuscript; J.L.R., L.F.C., A.S., and N.S.F. edited and revised manuscript; J.L.R., A.S., and N.S.F. approved final version of manuscript.

REFERENCES

1. **Zemanova MA**, **Knight A**, **Lybæk S.** Educational use of animals in Europe indicates a reluctance to implement alternatives. *ALTEX* 38: 490–506, 2021. doi:10.14573/altex.2011111.
2. **Wallace MJ**, **Zecharia A**, **Guilding C**, **Tucker S**, **McFadzean I.** Developing a new undergraduate pharmacology core curriculum: the British Pharmacological Society Delphi Method. *Pharmacol Res Perspect* 9: e00832, 2021. doi:10.1002/prp2.832.
3. **British Pharmacological Society.** Curriculum for the use of research animals. 2021. https://www.bps.ac.uk/education-engagement/research-animals/curriculum-for-the-use-of-research-animalshttps://

www.bps.ac.uk/education-engagement/research-animals/curriculum-for-the-use-of-research-animals.
4. **Marley SA**, **Siani A**, **Sims S.** Real- life research projects improve student engagement and provide reliable data for academics. *Ecol Evol* 12: e9593, 2022. doi:10.1002/ece3.9593.
5. **Connell GL**, **Donovan DA**, **Chambers TG.** Increasing the use of student-centered pedagogies from moderate to high improves student learning and attitudes about biology. *CBE Life Sci Educ* 15: ar3, 2016. doi:10.1187/cbe.15-03-0062.
6. **Wilson-Sanders SE.** Invertebrate models for biomedical research, testing, and education. *ILAR J* 52: 126–152, 2011. doi:10.1093/ilar.52.2.126.
7. **Aryal B**, **Lee Y.** Disease model organism for Parkinson disease: *Drosophila melanogaster*. *BMB Rep* 52: 250–258, 2019. doi:10.5483/BMBRep.2019.52.4.204.
8. **Reiter LT**, **Potocki L**, **Chien S**, **Gribskov M**, **Bier E.** A systematic analysis of human disease associated gene sequences in *Drosophila melanogaster*. *Genome Res* 11: 1114–1125, 2001. doi:10.1101/gr.169101.
9. **Hope KA**, **Johnson D**, **Miller PW**, **Lopez-Ferrer D**, **Kakhniashvili D**, **Reiter LT.** Transcriptomic and proteomic profiling of glial versus neuronal Dube3a overexpression reveals common molecular changes in gliopathic epilepsies. *Neurobiol Dis* 141: 104879, 2020. doi:10.1016/j.nbd.2020.104879.
10. **Ashmore LJ**, **Hrizo SL**, **Paul SM**, **Van Voorhies WA**, **Beitel GJ**, **Palladino MJ.** Novel mutations affecting the Na, K ATPase alpha model complex neurological diseases and implicate the sodium pump in increased longevity. *Hum Genet* 126: 431–447, 2009. doi:10.1007/s00439-009-0673-2.
11. **Lassuthova PR**, **Ravenscroft G**, **Lamont PJ**, **Davis MR**, **Manganelli F**, **Feely SM**, **et al.** Mutations in ATP1A1 cause dominant Charcot-Marie-Tooth type 2. *Am J Hum Genet* 102: 505–514, 2018. doi:10.1016/j.ajhg.2018.01.023.
12. **Yuan Y**, **Yu L**, **Zhuang X**, **Wen D**, **He J**, **Hong J**, **Xie J**, **Ling S**, **Du X**, **Chen W**, **Wang X.** *Drosophila* models used to simulate human ATP1A1 gene mutations that cause Charcot-Marie-Tooth type 2 disease and refractory seizures. *Neural Regen Res* 20: 265–276, 2025. doi:10.4103/1673-5374.391302.
13. **Haq IU**, **Snively BM**, **Sweadner KJ**, **Suerken CK**, **Cook JF**, **Ozelius LJ**, **Miller C**, **McCall WV**, **Whitlow CT**, **Brashear A.** Revising rapid-onset dystonia-parkinsonism: broadening indications for ATP1A3 testing. *Mov Disord* 34: 1528–1536, 2019. doi:10.1002/mds.27801.
14. **Montero-Pau J**, **Gómez A**, **Muñoz J.** Application of an inexpensive and high-throughput genomic DNA extraction method for the molecular ecology of zooplanktonic diapausing eggs. *Limnol Oceanogr Methods* 6: 218–222, 2008. doi:10.4319/lom.2008.6.218.
15. **Sekiya M**, **Iijima KM.** Phenotypic analysis of a transgenic Drosophila model of Alzheimer's amyloid-β toxicity. *STAR Protoc* 2: 100501, 2021. doi:10.1016/j.xpro.2021.100501.
16. **Fischer FP**, **Karge RA**, **Weber YG**, **Koch H**, **Wolking S**, **Voigt A.** *Drosophila melanogaster* as a versatile model organism to study genetic epilepsies: an overview. *Front Mol Neurosci* 16: 1116000, 2023. doi:10.3389/fnmol.2023.1116000.
17. **Koemans TS**, **Oppitz C**, **Donders RAT**, **van Bokhoven H**, **Schenck A**, **Keleman K**, **Kramer JM.** *Drosophila* courtship conditioning as a measure of learning and memory. *J Vis Exp* 124: 5608251, 2017. doi:10.3791/55808.
18. **Shiraiwa T**, **Carlson JR.** Proboscis extension response (PER) assay in *Drosophila*. *J Vis Exp* 3: 193, 2007. doi:10.3791/193.
19. **Pfeiffenberger C**, **Lear BC**, **Keegan KP**, **Allada R.** Locomotor activity level monitoring using the *Drosophila* Activity Monitoring (DAM) System. *Cold Spring Harb Protoc* 2010: pdb.prot5518–1101, 2010. doi:10.1101/pdb.prot5518.
20. **Koza Z**, **Ayajuddin M**, **Das A**, **Chaurasia R**, **Phom L**, **Yenisetti SC.** Sexual dysfunction precedes motor defects, dopaminergic neuronal degeneration, and impaired dopamine metabolism: insights from *Drosophila* model of Parkinson's disease. *Front Neurosci* 17: 1143793, 2023. doi:10.3389/fnins.2023.1143793.
21. **Slabach BL**, **Cooper RL.** An active learning approach to teach aspects of human dietary health using fruit flies as a model. *Adv Biol Lab Educ* 42: 39, 2022.
22. **Rand MD**, **Tennessen JM**, **Mackay TF**, **Anholt RR.** Perspectives on the *Drosophila melanogaster* model for advances in toxicological science. *Curr Protoc* 3: e870, 2023. doi:10.1002/cpz1.870.
23. **McCauslin CS**, **Gunn KE**, **Pirone D**, **Staiger J.** qPCR for second year undergraduates: a short, structured inquiry to illustrate differential gene expression. *Biochem Mol Biol Educ* 43: 273–282, 2015. doi:10.1002/bmb.20870.
24. **Mehta M**, **Singh A.** Real-time quantitative PCR to demonstrate gene expression in an undergraduate lab. *University of Dayton Biology Faculty Publications* 237, 2017. https://ecommons.udayton.edu/bio_fac_pub/237/?utm_source=ecommons.udayton.edu%2Fbio_fac_pub%2F237&utm_medium=PDF&utm_campaign=PDFCoverPages.