INQUIRY & INVESTIGATION

Drowsy *Drosophila*: Rapid Evolution in the Face of Climate Change

RECOMMENDED FOR *AP Biology*

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Abstract

Climate change can drive evolution. This connection is clear both historically and in modern times. The three-lesson curriculum described below provides opportunities for students to make connections between climate change and evolution through various modes of inquiry and self-investigation. Students examine how genetic variation may either facilitate or limit the ability for species to survive changing climates through work with the model organism Drosophila melanogaster. *Students are asked to layer new understanding of the mechanisms of evolution onto their observations of genetic variation in fruit fly thermotolerance, and then synthesize this information to make predictions regarding the survival of species threatened by climate change.*

Key Words: *climate change; evolution;* Drosophila melanogaster*; thermotolerance.*

○ Introduction

Climate change is an important and timely topic that unites many concepts in the biology curriculum. A recent study of 1500 public middle- and high-school science teachers from all 50 U.S. states found that although most students will be exposed to climate change in a science class, the average teacher devotes only one to two hours of instructional time to the topic (Plutzer et al., 2016). With so little time devoted to climate change education, teachers are missing a valuable opportunity to use climate change as a unifying concept to educate students about the nature of science and evolution. Despite the fact that climate change is driving both ecological and evolutionary change in contemporary populations, many teachers cover these topics as separate, discrete units. As classroom teachers, we felt this unconnected, multiunit approach could result in student misconceptions and a general lack of understanding about the importance of climate change on evolution. We emphasize that climate change can result in rapid, measurable, real-time evolutionary events. Many scientific studies show a range of species are experiencing genetic changes in response to recent, rapid climate change (Bradshaw & Holzapfel, 2006; Hoffmann & Sgro, 2011; Penuelas et al., 2013; Scheffers et al., 2016). Unfortunately, this perspective of rapid modern evolution is not covered in most classrooms.

Climate change is an important and timely topic that unites many concepts in the biology curriculum.

In this three-lesson curriculum unit (Table 1), we strive to develop student understanding of the impact climate change can have on microevolution in contemporary populations using the model organism *Drosophila melanogaster*. The objective of this curriculum is to unify teaching of climate change and evolution by providing students opportunities to assess natural genetic variation for cold tolerance in *D. melanogaster* and discuss the implications for genetic variation to allow for adaptation by natural selection and species persistence despite a changing climate.

We open with a data-collecting activity using species "information trading cards" to give students a broader understanding of climate change impacts on various species. Students discover that not all species have the capacity to survive rapid climate change. These cards illustrate climate change "winners" and "losers," and emphasize the critical concept that some species may benefit from climate change by expanding their ranges, population sizes, or number of generations per year, whereas other species may suffer more with reduced ranges, decreased population sizes, and even extinction (Foden et al., 2013). In the second lesson, students perform a laboratory investigation using genetically defined lines of the fruit fly *D. melanogaster* to investigate genetic variation in climate-change-related phenotypes. Students relate their laboratory observations to the mechanisms of evolution and perform statistical analyses. In the third lesson, we extend our discussion of climate change driving natural selection using a series of examples that highlight the diversity of species' responses to climate change and reinforce students learning the forms of natural selection: directional, stabilizing,

The American Biology Teacher, Vol. 80, No 4, pages. 272–277, ISSN 0002-7685, electronic ISSN 1938-4211. DOI: https://doi.org/10.1525/abt.2018.80.4.272.

Table 1. A suggested pacing guide that can be used with the curriculum based on 45-minute class periods. Since the classroom teacher knows his or her students best, the teacher should decide the sequencing of lessons. The curriculum is available at https://www.cpet.ufl.edu/resources/curricula/.

	Week 1	Week 2
Day 1	**Homework Prior to Lesson ONE:** Background article reading with guided questions. Administer pre-assessment (if using).	**Lesson TWO (extension):** Hardy-Weinberg extension lesson and practice set.
Day 2	**Lesson ONE:** Winners and Losers of Climate Change— Debrief background reading guide. Complete Winners and Losers activity with species cards; debrief.	**Lesson THREE:** Patterns of Natural Selection— Types of Selection lesson and practice.
Day 3	**Lesson TWO:** Chill Coma Assay and Evolution Investigation— Assay background presentation. Run assay; collect raw data.	**Lesson THREE:** Patterns of Natural Selection— Types of Selection lesson and practice.
Day 4	**Lesson TWO:** Chill Coma Assay and Evolution Investigation— Data analysis and lab wrap-up questions.	Administer post-assessment.
Day 5	**Lesson TWO:** Chill Coma Assay and Evolution Investigation— Mechanisms of Evolution student investigation pages.	

and disruptive selection. Students are asked to synthesize their understanding of evolution and climate change to make predictions about the survival of species currently threatened by climate change. Taken together, these three lessons provide students the opportunity: (1) to learn that species may differ in their responses to climate change; (2) to assess natural genetic variation for cold hardiness, a climate-related trait in *D. melanogaster*; and (3) to discuss the implications for genetic variation to allow for adaptation by natural selection and species persistence despite a changing climate.

Drosophila melanogaster as a Model Organism for Studying Climate Change

One aim of the *Next Generation Science Standards* is to more closely align science teaching and scientific processes. Thus, students must be exposed to the concept that some organisms can serve as models for much broader groups of organisms or for a set of scientific topics. Models are representations of complex phenomena that help students understand content, and do so in a way consistent with what scientists actually do in both laboratory and field settings (Bryce et al., 2016). Expanding the concept of using models, we found that *D. melanogaster* as a model organism was a good way to represent the larger biological phenomena of evolution and responses to climate change. *D. melanogaster* and other *Drosophila* species have served as models for thermal biology for decades, covering concepts from genetics of high-temperature responses to seasonal ecology, where they have often been used to model effects of climate change. Fruit flies are frequently chosen as a model organism for scientific studies because they have short life cycles, are more cost-effective than many other organisms (e.g., mice), and are easy to care for with limited space and effort. These same characteristics also make them ideal for classroom use.

D. melanogaster is a world-wide invasive species spread by people in stored foods and refuse that now occurs on almost every continent (David et al., 2007; Keller, 2007). This small fly originated in equatorial Africa, an area characterized by limited seasonality and generally warm temperatures. As the fly spread into more seasonal environments in the temperate zones of both the northern and southern hemispheres, populations have undergone rapid adaptation for cold hardiness and other seasonal traits (David et al., 1998; Hoffmann et al., 2002; Schmidt & Paaby, 2008). Thermal hardiness traits show a strong heritable genetic component across populations of *D. melanogaster*, as well as other invertebrates, along latitudinal clines from tropical to temperate locales consistent with directional natural selection (David et al., 2003; Gibert & Huey, 2001; Hoffmann, 2010; Sinclair et al., 2012).

Although multiple axes of cold hardiness differ among *Drosophila* populations, one of the most commonly measured traits which varies with latitude is chill coma recovery time. At low temperatures (below 4–7°C), insects and other ectotherms lose coordinated movement, resulting in an inactive state known as a "chill coma" (Sinclair et al., 2012). Flies sampled from populations originating in seasonally cool temperate locales typically recover more quickly from cold exposure than flies from warmer tropical locales (David et al., 2003; Gibert & Huey, 2001; Hoffmann, 2010; Sinclair et al., 2012). Because flies in chill coma cannot move to disperse, find mates, court, feed, or avoid predators, it is thought that the ability to recover quickly from chill coma is adaptive in seasonal habitats where flies may experience cold temperatures overnight that warm quickly in the morning. Chill coma recovery time has a clear, heritable genetic basis with polygenic quantitative patterns of inheritance. Individual genotypes within many populations clearly differ in this trait, showing that there is substantial naturally segregating variation in chill coma recovery time (Hoffmann et al., 2002; Morgan & Mackay, 2006; Norry et al., 2008; Mackay et al., 2012).

○ Methods

Chill Coma Assay Design

This curriculum was developed in collaboration with a university researcher to bring an existing research protocol, known as the chill coma recovery time assay, from the field of evolutionary thermal biology to the high school classroom. In the chill coma assay protocol, vials containing approximately 10 flies from six genetically distinct lines of *D. melanogaster* (for a total of 60 flies) are placed on ice for three hours to induce a chill coma. The recovery times of flies, defined as a fly's ability to walk, from each line are then measured.

Fly lines were taken from the *Drosophila* Genetic Reference Panel (DGRP, Mackay et al., 2012). Each line was produced from a separate, individually housed, wild-caught gravid female from a single population of flies in Raleigh, North Carolina. Offspring of each individual female were inbred >30 generations, producing >200 true-breeding isofemale lines (Mackay et al., 2012). True-breeding isofemale lines are useful for studying the role of genetic variation in phenotypic variation because allelic variants become fixed within each line, but differ between lines. Differences in chill coma recovery times among DGRP lines indicate substantial genetic variation for chill coma recovery in the original population (Mackay et al., 2012). Thus, there is potential for natural selection to act on this trait in response to contemporary climate change.

These lines can be ordered from the Bloomington *Drosophila* Stock Center (University of Indiana; http://flystocks.bio.indiana.edu/Browse/RAL.php). We chose lines that recovered quickly (7–12 minutes, 25186/RAL-360, 25198/RAL-555, and 28178/RAL-356) and lines that recovered slowly (>20 minutes, 28253/RAL-861, 28254/RAL-879, and 28260/RAL-897) to maximize phenotypic differences students would observe. We suggest these lines to others, but instructors could select from any number of lines with differences in chill coma recovery time or other phenotypes of interest (Mackay et al., 2012).

Lesson ONE: The Winners and Losers of Climate Change

In Lesson ONE, students addressed the key question, "Are all species equally affected by climate change?" Students were assigned two homework articles to prepare for a class activity involving climate change "winners" and "losers." In the article "More Evidence that Global Warming is Intensifying Extreme Weather" (Abraham, 2015), students learned how climate change is projected to produce not only higher average temperatures, but also greater frequency of extreme weather events including both heat waves and cold snaps. This article confronts a common misconception that climate change results only in rising temperatures. In the second article, "Evolutionary Response to Rapid Climate Change" (Bradshaw & Holzapfel, 2006), students learned about phenotypic plasticity, the ability of one genotype to produce more than one phenotype when exposed to different environments, and examined several examples of genetic changes that have already occurred in species due to climate change. Following a class discussion of the pre-reading, students worked in small groups (2–4 students) to analyze eight climate-affected species cards (included in the curriculum) and predicted which species populations are likely to increase ("winner") or decrease ("loser") in response to climate change trajectory. The cards are based on authentic data available from a study that analyzed species vulnerable to climate change (Foden et al., 2013). Students examined traits like dispersal ability, interspecific interactions, and habitat needs. Using additional information provided in a figure from Foden and colleagues (2015), students made a prediction about each species' ability to benefit or struggle due to climate change (Figures 1 and 2). This activity can correct two common misconceptions: (1) evolution only occurs over very long, geologic time periods; and (2) climate change will negatively affect all species. As an extension, students could conduct their own research and create new climate-affected species cards. Students could then trade cards with other groups and add new species to their Species Vulnerability Matrix.

Lesson TWO: Chill Coma Assay and Evolution Investigation

After students learned to identify species' vulnerability to climate change, Lesson TWO challenged students to determine the role of evolution and natural selection in the long-term survival of a

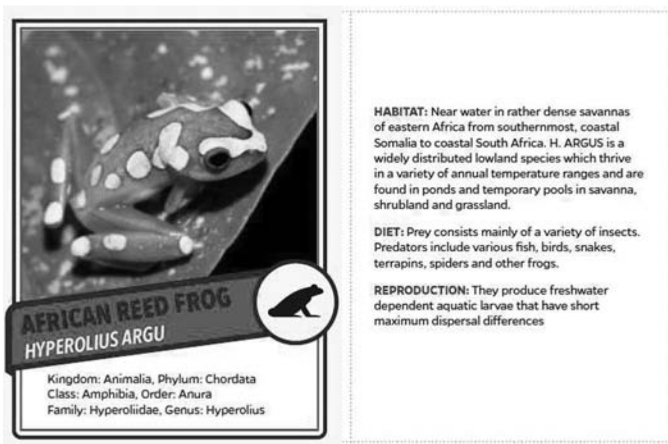


Figure 1. Students analyzed information provided on species cards to complete a matrix to assist in the prediction of species survival based on current climate change trajectories (see Figure 2).

SPECIES VULNERABILITY MATRIX: Check any of the following boxes for each species if the factor is contributing negatively towards the species continued success given the current impact of climate change. Column "e. rarity" has been completed for you, for all 8 species.

	a. specialized habit	b./c. environmental tolerances/ dependence on environmental triggers	d. interspecific interaction dependence	e. rarity	f. poor dispersal ability	
African Reed Frog						
Asian Tiger Mosquito						
Four Toed Lizard				x		
Coral				x		
Fantail Warbler						
Common Coqui				x		
Fingered Poison Frogs				x		
Hornbill						

Figure 2. Students determined vulnerability to extinction due to climate change using this matrix (adapted from Foden et al., 2013).

species. Lesson TWO is a multiple-day activity in which students conducted the chill coma recovery time assay and performed basic statistical analyses of results to answer the driving question, "Is there potential for natural selection to act upon chill coma recovery in *D. melanogaster*?" This lesson is engaging and, importantly, models real scientific research because students performed the authentic chill coma recovery time assay with live *D. melanogaster* specimens.

Students again worked in small groups to measure chill coma recovery time on one line of flies. As a class, a standardized procedure was developed based on the authentic protocol so group results could be accurately compared. At the conclusion of the experiment, class data were compiled to include replicates. Students created graphs of the mean recovery time for each of the six genetically distinct lines, and included standard errors of the means. A class discussion of the data was held in which students identified that the six genetically distinct fly lines vary in chill coma recovery time, indicating that chill coma recovery time has a genetic basis and is a trait upon which natural selection could occur.

Next, students explored the mechanisms of evolution via self-investigation. Students read passages discussing five classic mechanisms of evolution: mutation, gene flow, non-random mating, genetic drift, and natural selection. Passages can be modified by the instructor to tailor the lesson to students' ability or prior knowledge. Students applied their new knowledge of evolution to further interpret the laboratory data via a post-lab question set that begins with basic knowledge questions and proceeds to more open-ended questions in which students made their own connections between the content and laboratory activity.

As an extension of the lab investigation and mechanisms of evolution lesson, AP-level students explored the Hardy-Weinberg principle to quantify evolutionary change in a population of hypothetical fruit flies. This activity further expanded the students' understanding of biostatistics using the Hardy-Weinberg equations and the chi-squared statistical test. At the conclusion of Lesson TWO, AP-level students could be required to write a formal lab report that includes a discussion in which students expound upon the statistical results of their experiment and make predictions about the ability of the fruit fly population to respond to climate change based on their newly acquired knowledge of evolutionary mechanisms.

Lesson THREE: Patterns of Natural Selection

The third lesson asked students to synthesize their understanding of microevolution and climate change to answer the essential question, "What patterns of natural selection might occur as a result of climate change?" using teacher-selected species from the Foden study. In this one-day lesson, students learned about three forms of natural selection: directional, stabilizing, and disruptive selection. Students completed a practice set with different population scenarios and predicted what form of natural selection the population could undergo. As a formative assessment, students worked in groups of three to four to complete the Natural Selection in the Face of Climate Change activity, where students identified how a real population of organisms might respond to climate change. Students received a fact sheet with a species description and a problem the species faces as a direct result of climate change. Groups drew graphs to represent the selection event, before and after the effects of climate change, and wrote an explanation of their predictions to support how species could possibly avoid extinction (c.f. Figure 3). Student groups presented

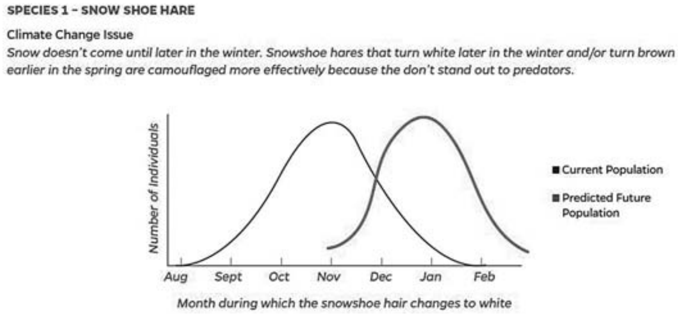


Figure 3. Sample answer key for a problem in the natural selection group modeling activity.

to the whole class, and students filled in a graphic organizer listing problems created by climate change and possible adaptations in response to those problems (Figure 4).

This lesson concluded with a class discussion on the limits of evolution: specifically, that species do not evolve because they need and/or want to, and that species are limited by existing genetic variation. We emphasized that evolution occurs at the population level, not the individual level. Finally, we discussed that the pace of evolution in a species may or may not keep up with environmental changes due to climate change.

○ Results

Student Outcomes

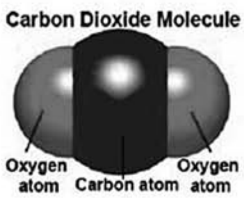
We implemented the curriculum in two different schools in AP Biology and honors-level biology classes. In the AP setting, this curriculum was used to begin the first unit on evolution. Students were asked to draw on their previous knowledge of evidence of evolution and basic genetic inheritance patterns from prior coursework. Students were previously introduced to statistical analysis in prior activities. In the honors-level class, the curriculum was used as a culminating piece, after the completion of a genetics unit, to tie microevolution to macroevolution, which had been introduced earlier in the course.

As teachers we observed higher levels of engagement using live model organisms compared to lessons from previous years using artificial representations of natural selection events (e.g., cut-out peppered moth activity). In addition, students made many positive comments about the curriculum on surveys. One stated: "I learned the most from this lab because it gave me a chance to find answers on my own, instead of just being told the answers." Another shared that "it was really cool to use live organisms and now I see how species might change because of climate change."

Modifications

For those who do not wish to or cannot use live fruit flies in the classroom, Lesson TWO could be approached as a case study, and students could be given data to analyze rather than collect their own. A data set is available from the authors. To better understand the impact of this curriculum on student understanding, a content knowledge assessment (available in the curriculum) and measure of evolution acceptance (GAENE, Smith et al., 2016) will be used as a repeated measure and allow comparison across classrooms in future studies.

CLIMATE CHANGE: *a change in global or regional climate patterns; attributed largely to the increased levels of atmospheric carbon dioxide produced by the use of fossil fuels.*

Problems Created By Climate Change	As a Result of Climate Change Natural Selection Would Favor*
	
1. Acclimation strategies become out of sync with changing seasons	*1. Snowshoe hares that turn white later in the winter and/or turn brown earlier in the spring*
2. Breeding ranges may shrink	*2. Baltimore Orioles that expand their range to higher latitude during the breeding season*
3. More rainfall and severe storms due to increased evaporation from warmer temperatures	*3. Juvenile Magellanic Penguins with more fat (to avoid hypothermia) and/or that develop adult feathers earlier in life*
4. Ocean acidification occurs when CO2 is absorbed by the ocean and creates carbonic acid	*4. Shellfish with thicker shells*
5. Loss of snowpack in mountainous regions	*5. Cascades tadpoles with a shorter larval period and/or are more resistant to desiccation*
6. Food sources may be out of sync with migration patterns	*6. Red knots that can find other food sources and/or arrive earlier in Delaware so arrival is synced with horseshoe crab egg laying*

Limitations to Evolutionary Adaptability:

1) *There may not be enough (or any) genetic variation in the population for a given trait*

2) *A population may not be able to evolve fast enough (especially organisms with longer generation times) to keep up with the pace of climate change*

Figure 4. Teacher key of completed graphic organizer from Lesson Three.

Conclusion

This curriculum facilitates diverse teaching and learning opportunities by combining science and mathematics and asking students to engage in critical thinking the concepts presented. Our goal was to have students engage in inquiry-based learning and naturally ask their own questions about the effects of climate change and the future of life on Earth. These questions will naturally touch on diverse topics including genetics, evolutionary biology, emerging pathogens, ecology, and the nature of science. Beyond science, it was our hope that students would also engage in cross-curriculum-based inquiry, especially involving the ongoing intersection of climate change science and the politics of climate change policy worldwide. By asking students to identify questions and concepts that guide scientific inquiry, and having them learn how to conduct a scientific investigation on their own, we believe students will develop a deeper understanding of concepts presented and be more prepared to educate themselves, their peers, and even their parents on topics of climate change and evolution.

For teachers interested in implementing this curriculum unit, all materials and detailed instructions can be found at: https://www.cpet.ufl.edu/resources/curricula/

○ Acknowledgments

Development of these materials was supported by funds from the Florida State Agricultural Experiment Station and the National Science Foundation (IOS- 1051890 and IOS- 1257298 to DAH). Additional thanks to Nicole DiLorenzo and Andre Szejner-Sigal who provided critical support in rearing and shipping *Drosophila* lines for classroom use.

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