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Lessons from Lizards: Adaptation to Introduced Ants

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ABSTRACT

The fence lizard and fire ant ecological system provides an excellent real-world case study for students to examine the impacts of nuisance introduced species on native organisms, with particular emphasis on the topic of adaptation. In this exercise, students are tasked with making predictions, analyzing real scientific data, and applying critical-thinking strategies to interpret their results. A reflection component at the end of the exercise involves the creation of a concept map to synthesize and integrate ideas from the lesson within the broader context of natural selection.

LEARNING OBJECTIVES

After this case study and exercise, students will be able to:
1. Identify the negative impacts that red imported fire ants can have on people and native organisms.
2. Predict and interpret the behavioral response of native lizards to attack by red imported fire ants.
3. Use evidence to explain how native fence lizard populations can respond, through different kinds of adaption processes, to the presence of red imported fire ants.
4. Apply statistical analysis to support an explanation that lizards with an advantageous trait tend to increase in proportion to those lacking this trait.

INTRODUCTION

William Buren described the red imported fire ant with the specific epithet name invicta, *meaning invincible, because of its resilient nature and the belief that it would be difficult to manage. The ant has proven to be appropriately named.*

- Department of Entomology and Plant Pathology, Auburn University

How can introduced species impact native organisms? Can populations of native organisms change in response to the establishment of introduced species? These important questions have implications for the conservation of ecological communities on scales from local to global, to which humans are intricately linked. In this module, you will engage and explore these questions and background concepts. Then you will take on the role of a research scientist working in the Southeastern United States and studying the ecology and behavior of native eastern fence lizards and red imported fire ants, an introduced species that is considered a nuisance. You will make predictions based on your observations, statistically analyze your data, and explain your results in the context of natural selection and adaptation.

PART A: ENGAGE AND EXPLORE

Background concepts: Natural selection

The purpose of this section is to generate curiosity and elicit responses that uncover what you know or think about natural selection. To begin, record your observations and any questions that come to mind in the graphic organizer below while watching this short video: https://youtu.be/X9VqF5xF8os?si=a0aLWpwGLaLcyrOC&t=3 (note: you might need to watch it more than once).

Provide as many detailed observations as possible in the *I notice* column and aim for at least three questions in the *I wonder* column.

I notice...	I wonder...
Example: something crawling on the lizard	*Example: what kind of lizard is that?*

Next, in small groups and using a large sheet of paper (or another approach recommended by your instructor), take about five minutes to create a drawing that illustrates *natural selection*—the process whereby organisms better adapted to their environment tend to survive longer and produce more offspring (Figure 1). The drawing can include labels, if necessary, but should have limited text. After five minutes, share and view the other groups' drawings. Discuss as a class what your group drew and the drawings you saw.

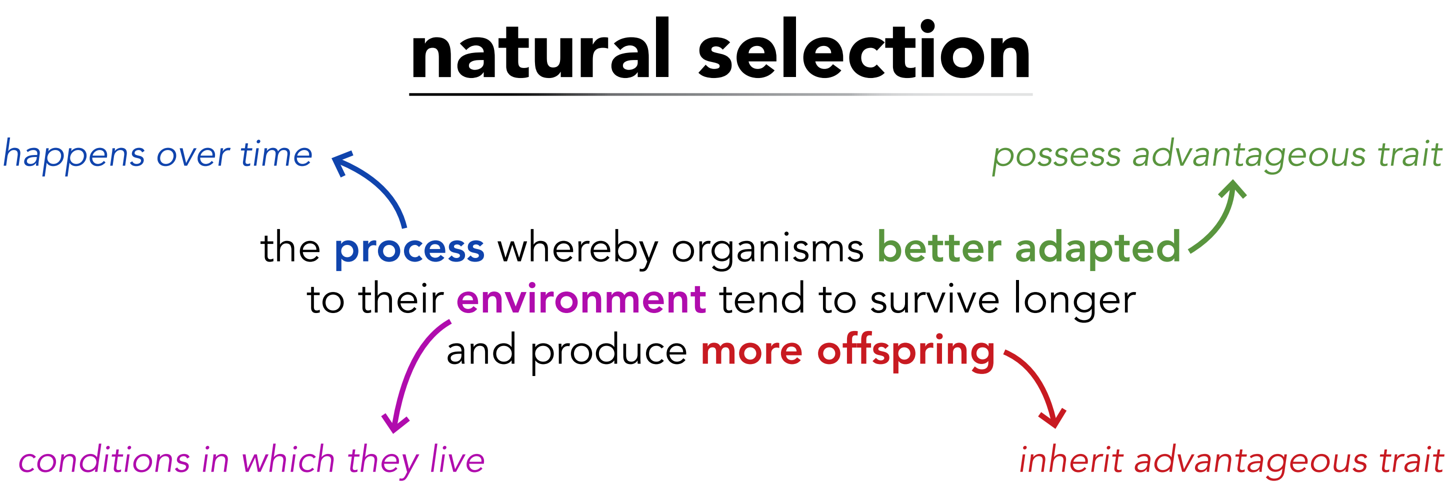

Figure 1. Expanded definition of *natural selection*.

Background concepts: Adaptation

Adaptation, as pointed out by Begon and colleagues (1996), is "a confusing word used to mean quite different things.

i. Characteristics of organisms evolved as a consequence of natural selection in its evolutionary past and which result in a close match with features of the environment and/or constrain the organism to life in a narrow range of environments.
ii. Changes in the form or behavior of an organism during its life as a response to environmental stimuli.
iii. Changes in the excitability of a sense organ as a result of continuous stimulation."

This case study and exercise explores adaptation as described in the first two definitions above. Focusing on definition (i) to start, an organism's evolved characteristics can be structural (physical), behavioral, or physiological (internal body process). Using the North American beaver (*Castor canadensis*) as an example, some of their evolved characteristics include:

- Webbed hind feet,
- Oil produced in special glands that they rub into their fur,
- Transparent inner eyelids,
- Ear and nose valves that close when submerged (e.g., under water), and
- Oxygen storage capacity in muscles is almost twice that of terrestrial mammals.

Discuss: Categorize each evolved characteristic as structural, behavioral, and/or physiological. How do you think these characteristics helped beavers, over time, survive better in their aquatic environment (e.g., ponds, wetlands)?

Elaborating on definition (ii) above, some organisms can respond behaviorally or physically to different environmental stimuli such as temperature. For example, the tree swallow (*Tachycineta bicolor*), a common and widespread bird species in northern North America, breeds earlier in warmer spring seasons (Dunn and Winkler 1999).

Discuss: What other environmental stimuli might trigger adaptive behavioral changes in an organism?

Species information: Eastern fence lizard

The eastern fence lizard (*Sceloporus undulatus*) is a medium-sized lizard (10–18.5 cm) native to eastern North America and occurs from the Atlantic coast to as far west as Texas (Conant and Collins 1998; Figure 2). Their average lifespan is uncertain but likely under five years. Belonging to the family of North American spiny lizards, eastern fence lizards have rough, pointed scales on their backs. They are gray to brown in color with a chevron pattern on their backs that can help them blend into their background. Most mature males, and some females, have patches of bright blue scales on their bellies and throats. Male eastern fence lizards establish and defend their territory by doing 'push-ups' to flash their blue scales and scare off other males.

Figure 2. Left: A juvenile eastern fence lizard (*Sceloporus undulatus*). Photo credit: Nicole Freidenfelds. Right: Can you spot the adult eastern fence lizard on the tree bark? Photo credit: Tracy Langkilde.

Eastern fence lizards are sit-and-wait predators that eat a wide variety of insects, spiders, and other invertebrates, including ants. They are common in many habitats, particularly open forests and field edges. Eastern fence lizards occasionally spend time on the ground foraging and moving between both natural and human-made basking/perch sites, such as tree stumps and tree trunks, rock piles, dead logs, and fence posts. Their cryptic coloration (i.e., camouflage) can reduce visual detection by some of their main predators including birds and domestic cats.

Species information: Red imported fire ant

Solenopsis invicta, commonly known as the red imported fire ant in the United States, is a species of fire ant native to tropical and subtropical South America; Figure 3). These ants thrive in ecologically disturbed areas and live in a wide variety of habitats such as rain forests, deserts, grasslands, alongside roads and buildings, and in electrical equipment (Callcott and Collins 1996). The red imported fire ant is one of the most successful nuisance species in parts of the world where humans accidentally introduced it: Australia, New Zealand, several Asian and Caribbean countries and the United States. See Box 1 for more information on introduced species.

Red imported fire ants have spread considerably throughout the southeastern United States since accidentally introduced into the port of Mobile, Alabama via cargo ship during the 1930s. They currently inhabit nearly 370 million acres in the U.S. and Puerto Rico. Counties throughout Texas, Arkansas, Oklahoma, Mississippi, Louisiana, Tennessee, Alabama, Georgia, Florida, South Carolina, and North Carolina are under Federal Quarantine, which restricts the interstate movement of regulated articles (e.g., hay, plants, soil) to prevent the human-assisted spread of fire ants (Figure 4).

Solenopsis invitca, like other ant species, perform valuable ecosystem functions such as nutrient turnover and soil modification, and serve as prey and detritivores within the system. As omnivores their diet consists of dead mammals, invertebrates (e.g., arthropods, earthworms), vertebrates, seeds, and sweet liquid substances from plants or honeydew-producing insects. In their native South American range, their abundance is moderated by competition with other ant species (Buren et al. 1974) and the presence of co-evolved predators (Porter et al. 1997). They often become the dominant ant species in introduced areas outside of their native range due to their aggressive foraging behavior, high reproductive capability and lack of predators and competitors (Allen et al. 2004).

Figure 3. Left: Red imported fire ants on a cotton swab. Right: Red imported fire ant mound in Covington County, Alabama. Photo credit: Nicole Freidenfelds.

Box 1: What's the problem with introduced species?

Introduced species are organisms non-indigenous to a specific region. While many introduced species are not destructive, some cause substantial damage to the ecosystem, economy, and/or public health in their new environment. There are several mechanisms through which introduced species can spread and establish themselves:

Introduction through human activities: This can happen accidentally through international trade, tourism, escape from captivity, or transportation. Organisms can "hitchhike" on goods, vehicles, or in ballast water from ships, or be introduced to an area by release of unwanted pets or live bait.

Intentional introduction: In some cases, species are deliberately introduced for specific purposes, such as agriculture, hunting, or ornament. However, these introduced species can become problematic if they have no natural predators, competitors, or pathogens in their new environment.

Climate change: As climates change, certain areas may become more hospitable to species that were previously limited by temperature or other factors. This can lead to species expanding their ranges and becoming problematic in new areas.

Lack of natural predators: Introduced species often lack natural predators or diseases that would normally keep their populations in check in their native ecosystems. This allows them to reproduce and spread rapidly in their new environment.

Rapid reproduction and adaptation: Introduced species often have high reproductive rates and adapt well to different conditions. This gives them a competitive advantage over native species in their new habitat.

Altered ecosystem dynamics: In some cases, introduced species can alter the ecosystem dynamics of their new habitat by outcompeting native species for resources like food, water, and shelter.

Where introduced, red imported fire ants can negatively impact people, agriculture, natural resources, and native organisms. They can damage crops by feeding on the buds and fruits of plants, especially corn, soybean, okra, and citrus. They sometimes chew through irrigation tubing and cause physical damage to other farm equipment from their mounds or through electrical shorts. A single fire ant can sting repeatedly, causing injury to, or killing livestock either intentionally to obtain prey or in fierce defense of their mound; young and newborn animals are especially susceptible to the ants' venom. All these actions can result in substantial economic losses. They can also harm wildlife by reducing native ant and other invertebrate biodiversity and injuring or killing native birds, reptiles, amphibians, and mammals (Allen et al. 2004). Because of their painful, venomous sting, red imported fire ants are a nuisance introduced species, particularly in urban areas, and can even cause allergic reactions including rare instances of anaphylactic shock in humans (Potiwat and Sitcharungsi 2015).

Species information: Fire ant-fence lizard interactions

These two species co-occur and overlap in habitat over much of the southern distribution of the fence lizards' range (Callcott and Collins 1996; Conant and Collins 1998; Langkilde 2009a). Fire ants build extensive underground tunnels to help them forage tens of meters away from their distinctive mounds, of which as many as 400 can be found on a single acre of land (Markin et al. 1975; Buhs

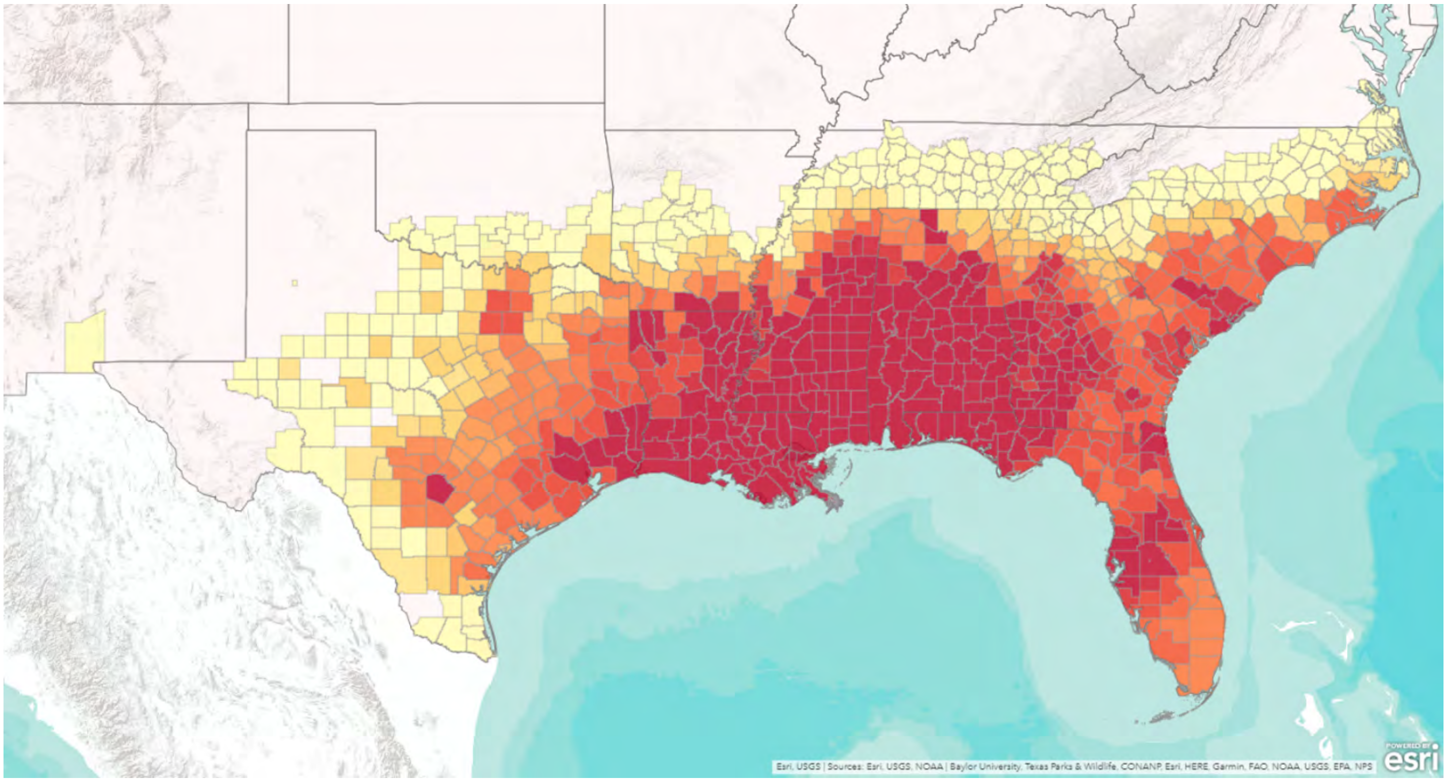

Figure 4. Map showing imported fire ant quarantined areas as of August 12, 2023. Color corresponds to the length of time since the quarantine was established in that county (dark = greater time). Visit the interactive online map (https://www.aphis.usda.gov/aphis/maps/plant-health/ifa-quarantine-mapping) to see if imported fire ants are expanding their range—are there new quarantine areas? You can also explore maps of other pests and diseases (https://www.aphis.usda.gov/aphis/newsroom/interactive-maps) surveyed by the United States Department of Agriculture Animal and Plant Health Inspection Service.

2005). Constantly using their efficient tracking capabilities to locate food sources, once fire ants find potential live prey, they use odor trails and chemical signals to quickly recruit other ants to join the attack (Vander Meer et al. 1988; Tschinkel 2006). This communication and cooperative behavior allows them to overcome and kill animals much larger than themselves, including fence lizards (Figure 5; also see: www.youtube.com/watch?v=F60agY1IpmU).

Researchers are studying how fence lizards and other native species respond to the threat of red imported fire ants. When fire ants and fence lizards come into contact, the lizards' behavior can determine whether or not they survive. Scientists discovered lizards that exhibit a behavioral response such as body twitching or fleeing can reduce their venom exposure and survive the attack. While it is possible that there exists a genetic basis to these behaviors and that lizard populations may adapt to exhibit higher frequencies of these behaviors, it is important to point out that not all behavioral responses are inherited and contribute to a population's evolution. As seen in adaptation's second definition (ii) above, some adaptive behaviors that improve an individual's longevity are environmentally induced and learned throughout an organisms' lifetime. Another factor to consider is that some behaviors may be adaptive and may be non-adaptive (i.e., counterproductive to an individual's survival or reproductive success), depending on the context or conditions.

Given the high frequency of encounters between fence lizards and red imported fire ants (Freidenfelds et al. 2012), there should be strong pressure for lizards to exhibit behavior that increases their survival. By understanding how animals at the edge of the fire ant inhabited area can protect themselves, scientists could aid the management of introduced species by providing insight into the

Figure 5. A red imported fire ant (*Solenopsis invicta*) attacking an eastern fence lizard (*Sceloporus undulatus*). The fire ant uses its mandibles to attach to the lizard and then pries up a scale to sting beneath it. Photo credit: Tracy Langkilde.

process and rate of behavioral adaptation both within and across generations of a population (e.g., Losos et al. 2004; Greenlees et al. 2010).

PART B: EXPLAIN

Key terms and misconceptions

Before continuing this exercise, read through and make sure you are familiar with these other key terms:

1. Advantageous: involving favorable circumstances that increase the chances of success.
2. Ecosystem: a biological community of interacting organisms and their physical environment.
3. Introduced Species: organisms non-indigenous to a specific region.
4. Native Species: a species that evolved to occupy a particular ecosystem without human assistance.
5. Nuisance Species: organisms that cause substantial damage to the ecosystem, economy, and/or public health, where introduced.
6. Population: group of organisms of the same species that live in the same area.
7. Proportion: a number considered in comparative relation to a whole.
8. Response: a reaction to something.
9. Statistically Significant: the likelihood that a relationship between two or more variables is caused by something other than chance.
10. Trait: distinguishing quality or characteristic.

Natural selection and adaptation are components of evolution, a complicated and often misunderstood process.

Pair and Share: Review and reflect on the misconceptions and realities about evolution highlighted in the table below.

Table 1. Common misconceptions, and corresponding realities, about evolution and evolutionary processes.

Misconception	Reality
Except for minor fluctuations from year to year, environmental conditions have stayed the same throughout the history of the earth.	The earth's history contains a rich past of significant and often dramatic changing environmental conditions and shifting land masses over millions of years.
Individual organisms can deliberately develop new heritable traits because they need them for survival.	Evolution does not occur through deliberate or conscious efforts of individual organisms. It operates through processes of variation, selection, and inheritance over long periods of time.
Sudden environmental change is required for evolution to occur.	While sudden environmental changes can sometimes drive rapid evolutionary responses, they are not a requirement for evolution to take place. Evolution is a continuous process that occurs in response to a variety of environmental conditions, both gradual and sudden.
Changes in a whole population cannot occur from the enhanced survival and reproduction of a few individuals.	Evolution is driven by the differential reproductive success of individuals with certain traits. This process of natural selection involves the preferential survival and reproduction of individuals with advantageous traits, leading to changes in the population over time.
Evolution happens when individual organisms acclimate or "get used to" new conditions gradually.	Evolution involves changes in the genetic makeup of populations over generations, not individuals over their lifetime, in response to environmental pressures.
Change occurs in the inherited characteristics of a population of organisms over time because of the use or disuse of a particular characteristic.	This "use/disuse" concept has been largely disproven by modern genetics. Evolution operates primarily through genetic variation, natural selection, and the accumulation of beneficial traits over generations.
Change to the characteristics of populations (i.e., the proportion of individuals in the population having certain traits) of organisms is always random and is not influenced by the favorability of that change in a given environment.	While variability in a population can arise by random chance, the process of evolution is driven by natural selection, where traits that enhance survival and reproduction in a given environment become more common over time. This results in a non-random change in the proportion of individuals with certain traits within a population.
Except for differences between males and females, and between young and old, all organisms of the same species look and act the same.	While individuals of the same species share certain common characteristics, there is inherent variation within populations due to genetic diversity that plays a crucial role in evolution and adaptation to changing environments.
The internal chemistry, appearance, and behavior of individuals of a species do not change, even over long periods of time.	Evolution is a continuous process that leads to changes in the internal chemistry, appearance, and behavior of individuals of a species over long periods of time.

Making predictions & analyzing results

Penn State biologist Dr. Tracy Langkilde conducts research on native fence lizards and their adaptive responses to the presence of red imported fire ants. The fire ants' well-documented spread through the southern U.S., and the fact that they are currently restricted to just a portion of the lizards' range, allows Dr. Langkilde's team to compare how lizards from ecologically similar areas differ behaviorally and physically based on the presence or absence of these fire ants.

Written Response: You have been given access to Dr. Langkilde's data in the associated Student Data Excel file provided by your instructor or downloaded from NCEP module collection at https:/ncep.amnh.org. Read the Part B research study notes below (Table 2). Work individually or in pairs through the comprehension questions and then make predictions and analyze the results using online statistical software.

Table 2. Research study notes (Part B) from Langkilde, T. 2009. Invasive fire ants alter behavior and morphology of native lizards. Ecology 90:208–217.

Overview	Adult fence lizards were placed on fire ant mounds for 60 seconds and their behavior was recorded. If a lizard ran off the mound (fled) before 60 seconds, the trial was ended at the time recorded. Lizards were closely monitored to make sure they did not get stung by too many ants.
Trial #	The experiment was conducted on 80 lizards total, 40 from each location.
Location	Alabama: Southern part of the state where fire ants were introduced in the 1930s. Arkansas: Eastern part of the state which has not yet been inhabited by red imported fire ants.
# Twitches	Number of times a lizard twitched its body while being exposed to fire ants for 60 seconds.
Time to Flee (sec)	Lizards that did not flee off the fire ant mound within 60 seconds were removed and scored as "61" (this is a conservative estimate because they may never have fled off the mound).

This study adhered to federal requirements for the humane care and use of animals in research and was approved by Yale University's Institutional Animal Care and Use Committee.

1. How many different locations were the fence lizards in this study from and where were they?
2. How long ago were fire ants introduced in Alabama?
3. How were *Time to Flee* data recorded for lizards that did not flee (run) off the fire ant mound in 60 seconds?
4. How many lizards were tested from each location?
5. Complete the prediction based on background information that you have previously been provided: Lizards from ________________ twitch ________________ than lizards from ________________ when exposed to red imported fire ants.

You will now test your prediction. Open the Excel file, click the *Data* tab and familiarize yourself with the information provided. (Note: you may use a spreadsheet software other than Microsoft Excel, but specific formula instructions or locations of functions may differ than what is provided here.)

To compare the two groups of lizards, those from Alabama versus those from Arkansas, you need to first calculate the average values for each. You do not need to do this with a calculator—Excel can do it for you. In the *Calculations* box under *Average # Twitches*, type the following for Alabama: =AVERAGE(C2:C41)

6. What is the average number of times that lizards from Alabama twitched in response to fire ants?

In the *Calculations* box under *Average # Twitches*, type the following for Arkansas: =AVERAGE(C42:C81)

7. What is the average number of times that lizards from Arkansas twitched in response to fire ants?

So what? Are these averages considered different from each other, or are they pretty much the same? Scientists will use statistical tests to support these types of determinations. You will now analyze the data using what is called an unpaired T-test. The unpaired T- test compares the averages of two groups.

8. What are the two groups you are comparing?
9. What variable did you calculate the average for?

Click the *GraphPad: unpaired T-test* link below the *Calculations* box in the Data tab of the Excel file. It should open the webpage shown in Figure 6 (left).

In the *1. Choose data entry format* box, make sure *Enter or paste up to 2000 rows* is selected.

In the *2. Choose a test* box, select *Unpaired t test*.

In the *3. Enter data box*, change the QuickCalcs data label text to *Alabama* and *Arkansas*. Then, in Excel, use your mouse to select the data in the *# Twitches* column (C) for only the Alabama lizards. Copy what you selected and paste it into the QuickCalcs box. Repeat for the Arkansas lizards. Your data should look like what is shown in Figure 6 (right).

Click the *Calculate Now* button.

Let's examine the results.

P value and statistical significance:

10. The two-tailed P value equals _____________.
By conventional criteria, this difference is considered to be ___________ statistically significant. What does this mean? A P value less than 0.05 means that the difference you observed would happen rarely due to random sampling, and most likely there is a difference that is large enough to be explained by the hypothesis you are testing. In short, the analysis tells us that there is a significant difference in the number of ___________ between the two groups of lizards.

You will now visualize the data graphically in Excel. Type the average number of twitches you calculated earlier for each group in the *Part B Summary* tab where appropriate. Your first graph should have been generated automatically. It already includes error bars that indicate the uncertainty

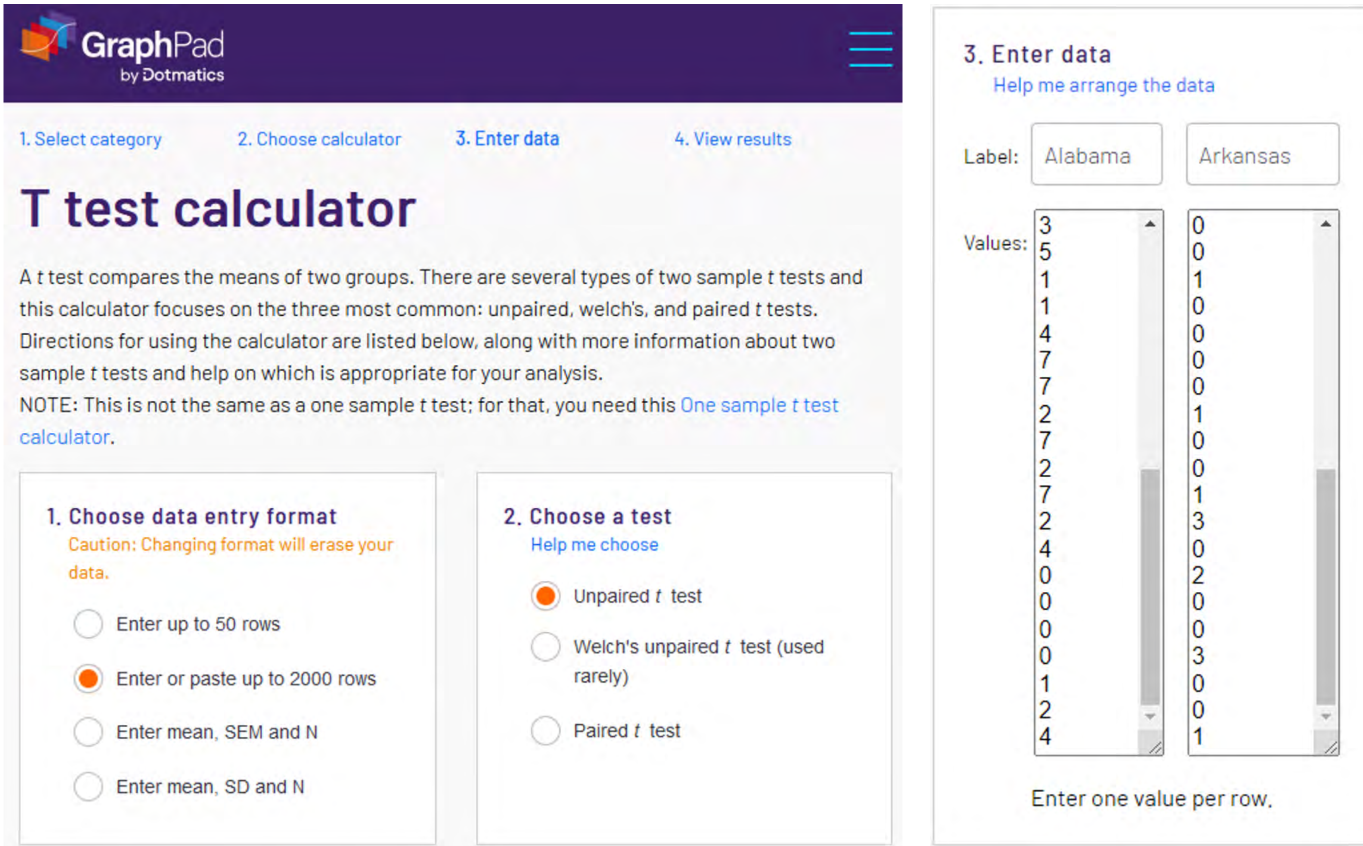

Figure 6. Screenshots of the GraphPad T test calculator webpage (https://www.graphpad.com/quickcalcs/ttest1/?Format=C).

in a reported measurement. Small error bars are good!

11. Describe the graph. Be as detailed as possible.
12. Was your earlier prediction correct? Explain why or why not.
13. Recall the second definition of adaptation in Part A: changes in the form or behavior of an organism during its life as a response to environmental stimuli. Why do you think the lizards from Alabama twitched more than those from Arkansas?
14. Now think about the data Dr. Langkilde measured for how long it took lizards to flee in response to fire ants. Make a prediction based on the background information that you have previously been provided: Lizards from ________________ flee _______________ than lizards from ________________ when exposed to red imported fire ants.

In the *Calculations* box under *Average Time to Flee*, type the following for Alabama: =AVERAGE(D2:D41)

15. What is the average time to flee for lizards from the Alabama site when exposed to fire ants?

In the *Calculations* box under *Average Time to Flee*, type the following for Arkansas: =AVERAGE(D42:D81)

16. What is the average time to flee for lizards from the Arkansas site when exposed to fire ants?
17. What unit is *Time to Flee* reported in?

Again, we need to analyze these values to see if they are statistically different. Highlight, copy and paste into QuickCalcs the same way you did before and then click the *Calculate Now* button.

P value and statistical significance:

18. The two-tailed P value is less than ________________.
    By conventional criteria, this difference is considered to be ____________ statistically significant.
    What does that mean? Write out a result statement in your own words.

Create a graph of the *Time to Flee* data by typing the average values into the appropriate boxes in the *Part B Summary* tab. The graph with error bars should have been generated for you (if you don't see it, scroll down!).

19. Describe the graph. Be as detailed as possible.
20. Was your prediction correct? Explain why or why not.
21. How many seconds faster, on average, did lizards from Alabama flee from fire ants than lizards from Arkansas?
22. Why do you think the lizards from Alabama fled faster than those from Arkansas?
23. What can happen if a lizard does not twitch or flee (run away) in response to fire ants?
24. Based on the data you analyzed, which population of lizards, those from Alabama or Arkansas, do you think would survive better in the presence of fire ants? Explain why.

Read the following excerpt modified from Tylan and Langkilde (2023, The Conversation).

This [twitching/fleeing] behavior is common in baby fence lizards, which are vulnerable even to native ants, but is usually lost in adults as they outgrow threats from native ants by getting larger. However, in areas with fire ants, adult lizards retain this behavior that better enables them to survive fire ant attack.

Dr. Langkilde's research suggest that the higher percentage of behaviorally responsive adult lizards in fire ant areas could be the result of selection acting against unresponsive adults (e.g., if you don't twitch, you have a higher chance of dying from fire ant attack, and hence reproducing) and/or it could also be due to lifetime exposure that leads lizards to benefit from continuing some of their juvenile behaviors into adulthood.

What this means is that the fence lizard's twitching behavior is complicated! It may be that natural selection acts on a heritable twitching trait across generations for all lizards, even those that haven't been exposed to fire ants, because babies that twitch are more likely to survive into adulthood and reproduce. And, as lizards that live where fire ants have been introduced grow into adults, they learn to "keep twitching" because that behavioral response removes attacking fire ants.

Tylan and Langkilde continue…

Lizards can't tell whether they have a potentially deadly fire ant crawling on them or if it's something harmless like a fly. So, to be safe, they respond in the same way to anything that they feel climbing on their scales. Unfortunately, this shake-it-off behavior doesn't solve all the lizards' problems, since it breaks their usual camouflage, making them more obvious to visual predators like birds. We have observed more evidence of wounds in fire ant-adapted lizards.

25. Do you think adult lizards from Alabama twitched and fled in response to fire ants when they were

first introduced to the area in the 1930s? Explain why or why not.
26. Under what conditions could twitching and fleeing be non-adaptive lizard behaviors?

PART C: ELABORATE

Beyond behavior

Watch the lizard video again: https://youtu.be/X9VqF5xF8os?si=a0aLWpwGLaLcyrOC&t=3.

Pair and Share: Which physical trait or characteristic is most important in helping lizards remove fire ants? Focus on physical (body) traits that are easily measurable.

Written Response: You will again be using Dr. Langkilde's data in the associated Student Data Excel file. Read the Part C research study notes (Table 3) and examine Figure 7 below. Then, work through the following questions individually or in pairs.

Table 3. Research study notes (Part C) from Langkilde, T. 2009. Invasive fire ants alter behavior and morphology of native lizards. Ecology 90:208–217.

Overview	Adult fence lizards were placed on fire ant mounds for 60 seconds and their behavior was recorded. If a lizard ran off the mound (fled) before 60 seconds lapsed, the trial was ended at the time recorded. Lizards were closely monitored to make sure they did not get stung by too many ants.
BL (cm)	Body Length: measure of lizard body length from tip of snout (nose) to base of tail (Figure 6)
HLL (cm)	Hind Limb Length: measure of lizard hind limb length (Figure 6)
REL HLL (cm)	Relative Hind Limb Length: longer lizards have longer hind limbs; to account for differences in lizard size, it is important to compare the relative hind limb length, that is the length of the hind limb as a ratio of body length (REL HLL = HLL / BL)

1. All the data you have analyzed up until now have been behavioral data—how the lizards from different populations responded behaviorally to fire ants. Think back to the video you just watched. What physical (body) trait would best help a lizard remove fire ants crawling on it? You may want to watch the video again.
2. There are two types of physical trait data that Dr. Langkilde measured for each lizard, BL and HLL. What do these abbreviations stand for?
3. Why was it important to calculate a relative hind limb length?
4. What is the formula that Dr. Langkilde used to calculate the lizards' Relative Hind Limb Length (REL HLL)?

In the *Data* tab, type the following formula into the first empty box of the REL HLL column of the data table: =F2/E2

Click that box and while holding down the mouse button, highlight the rest of the empty boxes in that column. Release the mouse button and press CTRL+D (or Command+D on Mac) to autofill the formula into the rest of the boxes. There should now be data in the entire table.

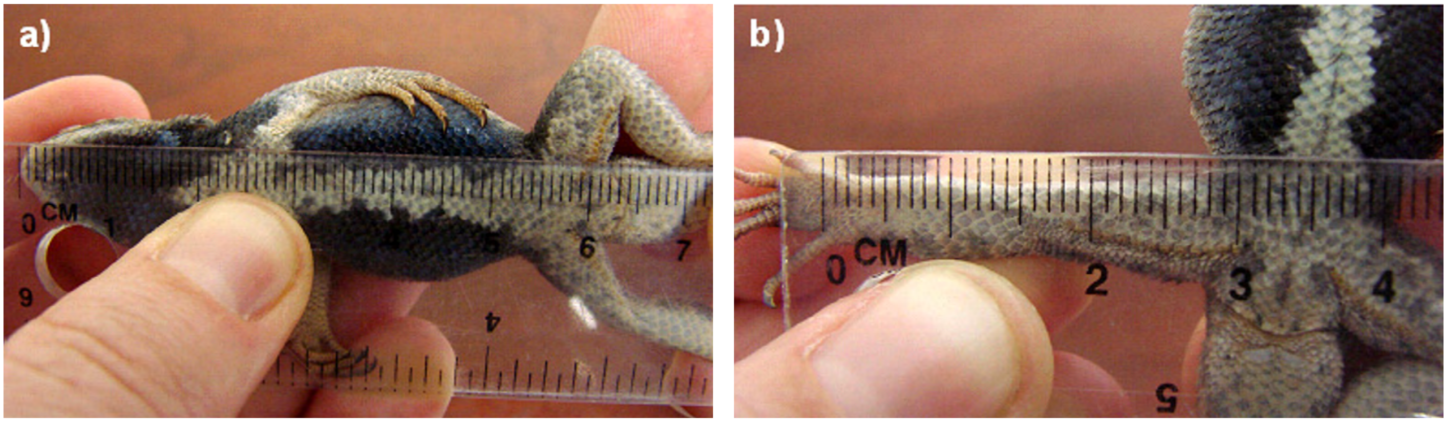

Figure 7. Demonstration of how eastern fence lizard a) Body Length and b) Hind Limb Length were measured in Dr. Tracy Langkilde's study. Photo credit: Nisha Ligon (photographer), Langkilde, T. 2009. Invasive fire ants alter behavior and morphology of native lizards. Ecology 90:208–217.

5. What unit of measure is REL HLL recorded in?
6. Make a prediction based on the background information that you have previously been provided: Lizards from ______________ have _____________ hind limbs than lizards from ______________.

In the *Calculations* box under *Average REL HLL*, type the following for Alabama: =AVERAGE(G2:G41)

7. What is the average relative hind limb length of lizards from Alabama?

In the *Calculations* box under *Average REL HLL*, type the following for Arkansas: =AVERAGE(G42:G81)

8. What is the average relative hind limb length of lizards from Arkansas?
9. Do these two values seem very different, yes or no? What is the mathematical (i.e., numerical) difference between them?

You will again perform an unpaired T-test to statistically compare the averages of the two groups. Highlight, copy and paste into QuickCalcs the same way you did before and then click the *Calculate now* button.

P value and statistical significance:

10. The two-tailed P value is less than _______________
    By conventional criteria, this difference is considered to be _________ statistically significant. How can a difference of only 0.013 cm be significant? For scale, most cell phones are slightly smaller than 1 cm thick.

Examine the data visually. Create a graph of the REL HLL data by typing the average values into the appropriate boxes in the *Part C Summary* tab. The graph with error bars should have been generated for you.

11. Describe the graph. Be as detailed as possible.
12. Was your earlier prediction correct? Explain why or why not.

It is hypothesized that there is a genetic basis to these differences in relative hind limb length. Read another excerpt from Tylan and Langkilde (2023, The Conversation):

We find lizard populations that have been living with fire ants have adapted [over generations] to have longer legs, which are better at removing fire ants when a lizard twitches and flees. This is a big shift for this species, reversing the latitudinal pattern we see in museum specimens—lizards tend to have shorter limbs the closer the population is to the equator.

The graph (Figure 8) shows data from the same two lizard populations in Dr. Langkilde's study. Use the graph and evidence from your statistical analyses from Part B and Part C to support the explanation that lizards with advantageous traits against fire ants tend to increase in proportion to those lacking these traits.

Putting the pieces together

Watch a brief video from Penn State Eberly College of Science for insights on additional research from Dr. Langkilde's team: "Lizards adapt to invasive fire ants, reversing geographical patterns in traits" (https://youtu.be/2jL6qOGV2BI).

Written Response: In pairs, create a concept map to illustrate how the fence lizard and fire ant system fits into the broader context of natural selection and adaptation (for guidance on concept maps, visit: https://scarfedigitalsandbox.teach.educ.ubc.ca/concept-maps-for-teaching-and-learning/ or https://creately.com/guides/how-to-create-concept-maps/).

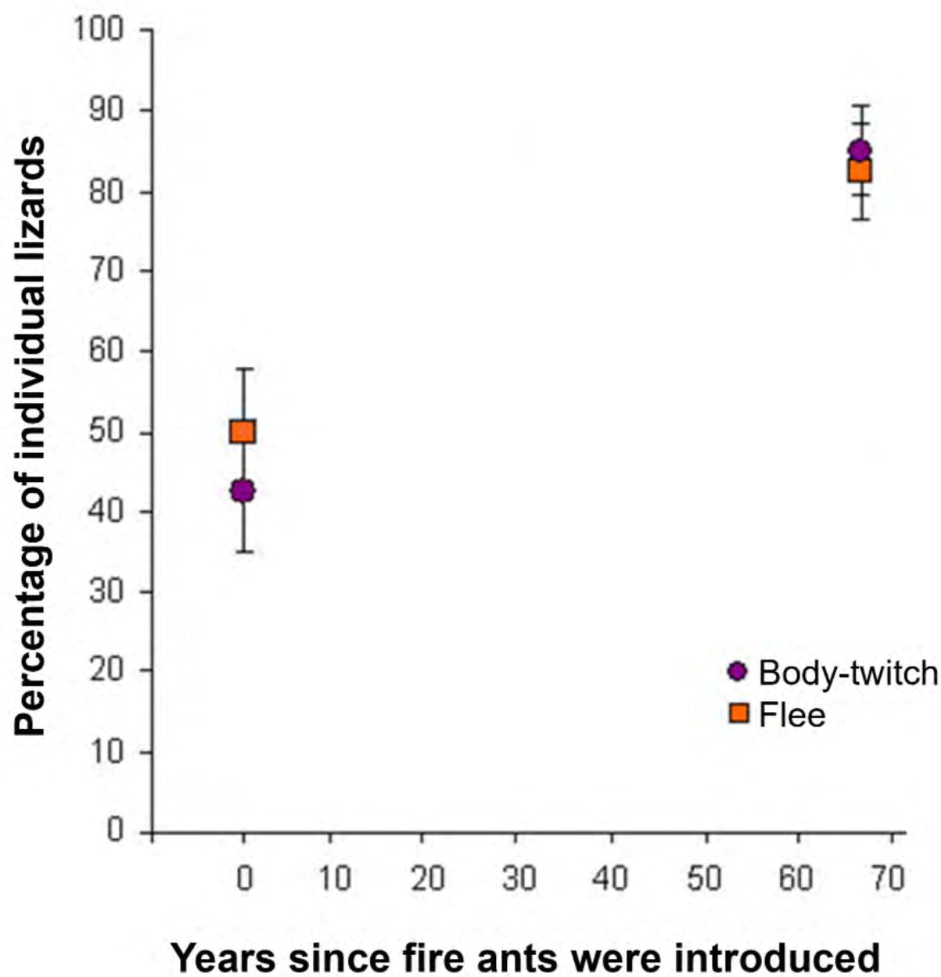

Figure 8. The proportion of eastern fence lizards that behaviorally responded during exposure to invasive fire ants at two sites with different time since invasion. Photo credit: Modified from Langkilde, T. 2009. Invasive fire ants alter behavior and morphology of native lizards. Ecology 90, 208–217.

PART D: EVALUATE

Final synthesis

Written Response: Your instructor might assign the following individual prompt as a form of assessment for this exercise: First, identify the negative impacts that introduced fire ants can have on people and native wildlife. Then, construct an explanation based on evidence for how native fence lizard populations are able to respond to red imported fire ants. Are there aspects of their response that you would consider a behavioral response that can develop within an individual's lifetime? Are there aspects of their response that you feel are compatible with adaptation via natural selection? Why?

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