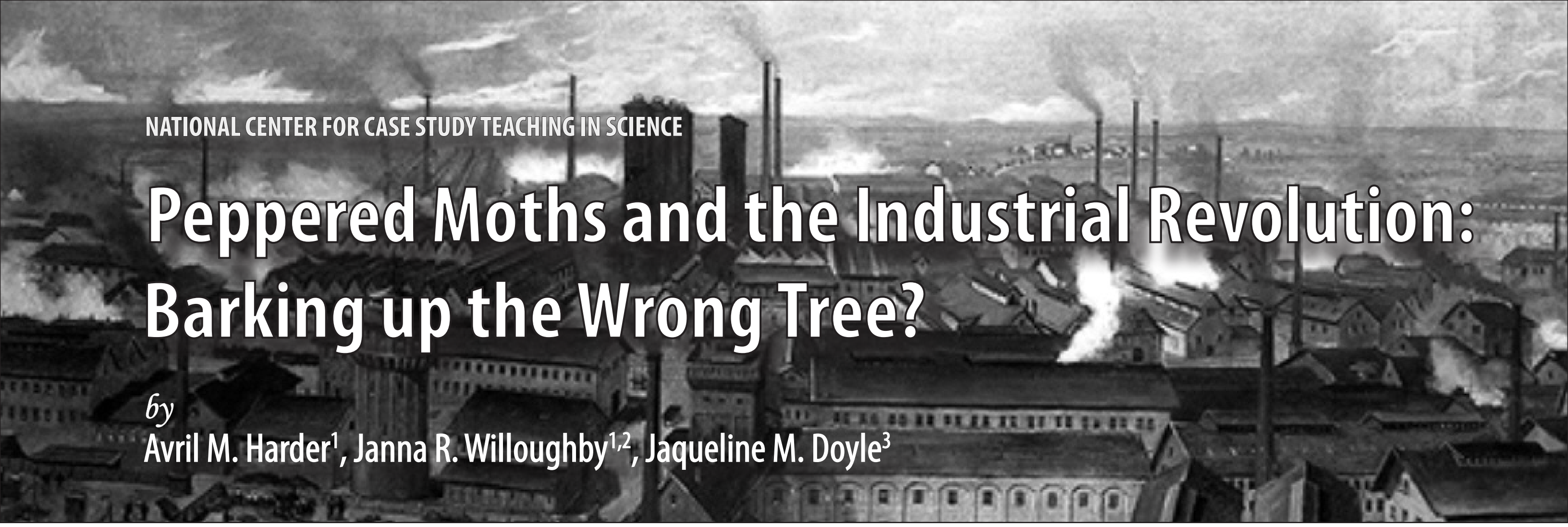

NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Peppered Moths and the Industrial Revolution: Barking up the Wrong Tree?

by
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Part I – Hypotheses and Predictions

The story of how Britain's Industrial Revolution led to changes in peppered moth coloration is a classic, textbook example of evolution in action. You might even already be familiar with the plot and the findings of the main researcher of the phenomenon, H.B.D. Kettlewell. Although the peppered moth story has been told and re-told in thousands of biology classrooms, most narratives leave out an important bit of history: after Kettlewell published his results that described changes in morph population frequencies over time, many of his peers questioned the validity of his data. Your first task in this case study will be to put yourself in the place of one of Kettlewell's contemporaries, evaluate his experimental methods, and decide whether his data support his assertions.

Background

Biston betularia is a medium-sized, nocturnal moth. Although the peppered moth is found across the world, some of the first research on this species was conducted in Great Britain. Before the Industrial Revolution, tree trunks in unpolluted areas were covered with lichens, and peppered moths with light colored wings were well camouflaged against this background (Figure 1). In this environment, light morphs vastly outnumbered dark morphs. During the Industrial Revolution, increasing air pollution led to the formation of acid rain and inhospitable conditions for the lichens. The death of the lichens combined with soot deposition on tree trunks led to an important environmental shift for the moths. The light morphs now clearly stood out from the newly darkened tree trunks, whereas the dark morphs had become camouflaged.

Figure 1. Dark and light peppered moth morphs resting on light-colored bark. *Credit:* Martinowksy, CC BY-SA 3.0, <https://bit.ly/2LMMpt1>.

Based on what you know about camouflage, predator avoidance and survival, hypothesize about how the moth population may have been impacted by environmental changes coinciding with the Industrial Revolution. Once you have defined your hypothesis and prediction (space provided below in Question 1, next page), plot your predicted population frequencies of the light and dark moth morphs over time in the figure below Question 2.

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Hypothesis Construction Guidelines

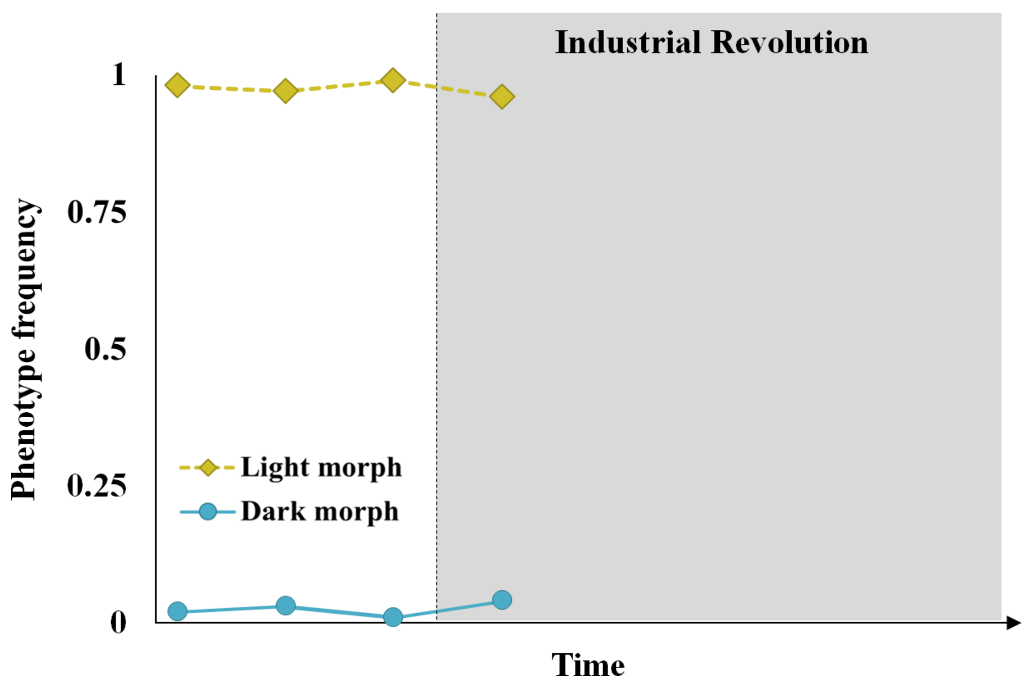
Figure 2. Little brown bat (*Myotis lucifugus*) with white-nose syndrome. The fungus (*Pseudogymnoascus destructans*) is visible as a white, powdery covering on the nose of the bat. *Credit:* Ryan von Linden/NY DEC, <https://bit.ly/2LKyZO3>.

Unsure how to write a testable hypothesis and prediction? Let's consider a quick example. White-nose syndrome (Figure 2) is a fatal fungal disease responsible for killing millions of bats in eastern North America. You know that bats often roost in colonies of 100 to 1000 individuals, and hypothesize that white nose is more easily transmitted when bats are roosting at high densities. You visit a number of caves and quantify colony size and the proportion of individuals with white nose syndrome at each site. You might predict that, in your data set, you will see a positive relationship between colony size and the proportion of bats with white nose syndrome. Your hypothesis and prediction can be combined into an "if-then" statement: if white nose is more easily transmitted at high bat densities, then the number of bats in a colony will be positively correlated with the proportion of bats with white-nose syndrome.

Questions

1. Define a hypothesis and prediction (if-then statement) regarding moths and the post-Industrial Revolution environment. Remember to consider camouflage and predator avoidance in your hypothesis (*if*) and morph frequencies in your prediction (*then*).

2. Using the graph below, plot your prediction of peppered moth morph frequencies over time.



Part II – Experimental Assumptions

Kettlewell began his work on peppered moths in the 1950s, 100 years after the first dark morph moth was recorded in Manchester, England in 1848. He collected moths at night using light traps, marked each moth, and released them back into the environment by placing the moths onto tree trunks during the day. He would later trap moths again and calculate the relative survival of each morph. The questions below are designed to help you identify some of the assumptions present in Kettlewell's design.

Questions

1. For each environment/phenotype combination, assume that Kettlewell released 100 moths. In the table below, predict and record how many moths you think Kettlewell recaptured for each morph and environment combination.

Environment	*Phenotype*	*Predicted number recaptured*
unpolluted / light bark	light	
unpolluted / light bark	dark	
polluted / dark bark	light	
polluted / dark bark	dark	

In his experiment, Kettlewell released 473 dark and 496 light moths into an unpolluted forest and later recaptured 30 dark (6% recaptured) and 62 light (12.5% recaptured) moths. Conversely in a polluted forest, when he released 154 dark and 64 light moths, he recaptured 82 dark (53% recaptured) and 16 light (25%) moths (Figure 3).

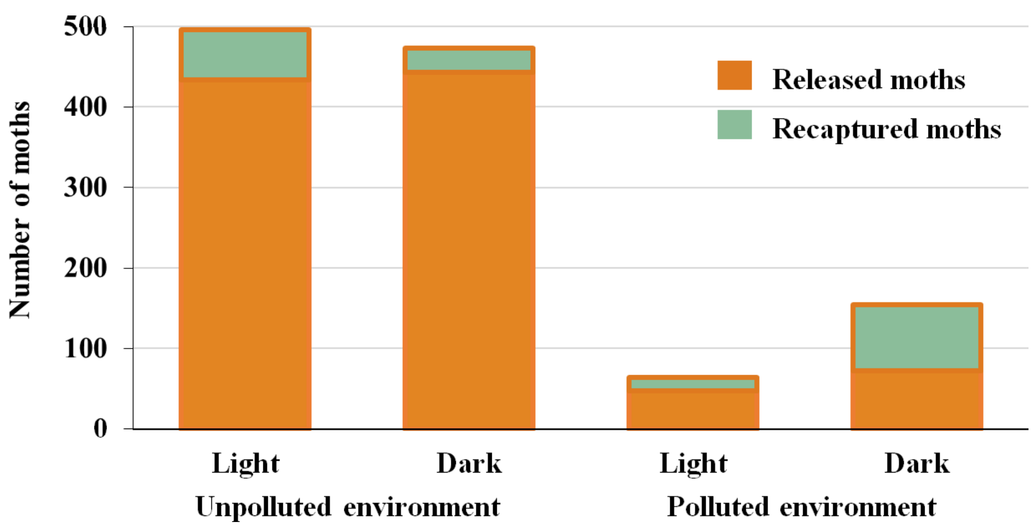


Figure 3. Results of Kettlewell's mark-recapture experiment for light and dark moth morphs released in unpolluted and polluted environments. In the unpolluted environment, light morphs were recaptured in larger numbers than dark morphs; in the polluted environment, dark morphs were recaptured in higher proportions than light morphs. Data from Kettlewell (1956).

2. Kettlewell interpreted these patterns as evidence for differential predation of the two morphs by birds, depending on whether the forest was polluted or not. Aside from predation by birds, what else could be leading to differential survival of morphs in polluted/unpolluted forests?

Improving on Kettlewell

Regardless of design, all experimental methods involve making certain assumptions. Some assumptions may safely be made. For example, if you are conducting an experiment that involves culturing *Escherichia coli* bacteria in a Petri dish, you could safely assume that marking the outside of the dish to distinguish your experimental groups from one another will not affect the growth of the bacteria inside the dish. However, if for the same experiment, you decided to use a custom, never-before-tested recipe for the bacterial growth medium, it would probably be a mistake to make assumptions concerning how well the bacteria will grow in that medium.

In Kettlewell's mark-recapture experiments, he made several important assumptions. For example, by placing moths on tree trunks randomly, he assumed that moths would not naturally seek out backgrounds that match their own coloration. Is it possible that a moth can detect differences between its own coloration and its background and that a moth will move to a surface that provides the moth with better camouflage?

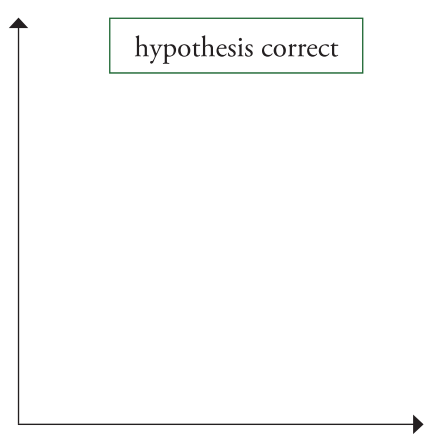
Questions

3. Imagine that you are going to improve upon Kettlewell's approach by addressing the assumption that moths randomly select tree trunks as resting spots during the day and do not attempt to camouflage themselves. What hypothesis would your experiment test, specifically? Use an if-then statement to outline your hypothesis and prediction.

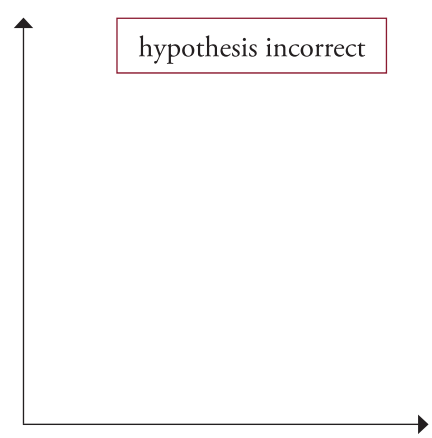
4. Now that you have defined the issue you would like to test and clearly stated your hypothesis and prediction, design your experiment. How would you experimentally test your hypothesis and the validity of your prediction? Don't worry about determining appropriate sample sizes or numbers of replicates—focus on describing an experimental approach to test the validity of your if-then statement.

5. Below, draw two figures that describe what you expect your results to be if (a) your hypothesis is correct and if (b) your hypothesis is incorrect. Be sure to label the axes of each figure.

a)



b)



Part III – Responding to Criticism

Many reviewers of Kettlewell's work were not convinced that birds would actually feed on moths that don't match their background more often than on moths that do match their background. In one publication, Kettlewell admitted that "… the editor of a certain journal was sufficiently rash as to question whether birds took resting moths at all," despite photographic evidence (Figure 4). Knowing that birds rely primarily on vision to locate food, Kettlewell designed visual acuity experiments to test whether birds exhibit feeding preferences with respect to moth color and background.

Figure 4. A spotted flycatcher (*Muscicapa striata*) approaching a dark peppered moth morph that is resting on an oak trunk. Photo from Kettlewell (1956). Reprinted by permission from Springer Nature.

First, to test the visual "conspicuousness" of the light and dark morphs, both moth morphs were placed on light and dark tree trunks (representing polluted and unpolluted environments), and a visibility score was assigned to each combination from varied distances by independent human observers. Your task is to evaluate Kettlewell's approach for testing this important assumption.

Questions

1. What did Kettlewell want to test when he designed this study?

2. What did Kettlewell actually test when he conducted this study?

3. Given the answers above, should Kettlewell expect the results of this experiment to determine the visibility of moths on varying backgrounds to birds (and therefore, likelihood of predation)? Why or why not?

True for Humans, True for Birds?

Human observers of light and dark morphs on varying backgrounds confirmed that, to humans, moths that matched their background were far less conspicuous than moths that did not. To test whether this was also true for birds, Kettlewell performed experiments with two birds housed in an aviary. This large cage was supported by dark tree trunks but also contained lighter trunks with lichens present. The birds were sequestered while moths of both morphs were released into the cage in equal numbers. The birds were then given free reign of the cage and their behavior was documented. Neither bird ate any moths in the first two hours of observation. In the third hour, all dark moths on light backgrounds and all light moths on dark backgrounds had been eaten, as well as a few moths that matched their backgrounds. The next day, Kettlewell repeated the experiment with the two birds, releasing equal numbers of light and dark morphs. After 30 minutes, all but two of the moths released had been eaten by the birds.

Questions

4. What might explain the differing results Kettlewell recorded between the two days of the experiment?

5. How would you alter Kettlewell's experimental design to test his research question: *do birds preferentially prey upon moths with respect to the moths' resting backgrounds?* Address the issue of sample size in your answer. Be prepared to share your designs with the class.

Part IV – Identifying the Agent of Selection

The key aspect that was missing from Kettlewell's experiments was the agent of selection—was bird predation the key to peppered moth color morph evolution or was this connection due to the various unresolved assumptions of his experiments? In the early 2000s, Michael Majerus set out to test, once and for all, whether bird predation was the selective agent in Kettlewell's forest experiments. Majerus died shortly after presenting his work at a 2007 conference, but his results were published by four colleagues in 2012. First, Majerus gathered observational data on 135 moths to determine where they naturally rest during the day. Next, Majerus conducted predation experiments. He only released moths at densities and morph frequencies mimicking natural values, releasing a total of 4864 moths over six years. Because moths are active at night, he released each moth into a netting "sleeve" placed around a tree branch at dusk, allowing moths to select their resting place at the ends of their nightly flight patterns. Before sunrise, the sleeves were removed from the branches. Four hours after sunrise, Majerus examined the tree branches and moths not present at their chosen resting places were presumed to have been eaten by birds. He tallied the proportion of light and dark morphs surviving over the course of the experiment in an unpolluted forest (Figure 5).

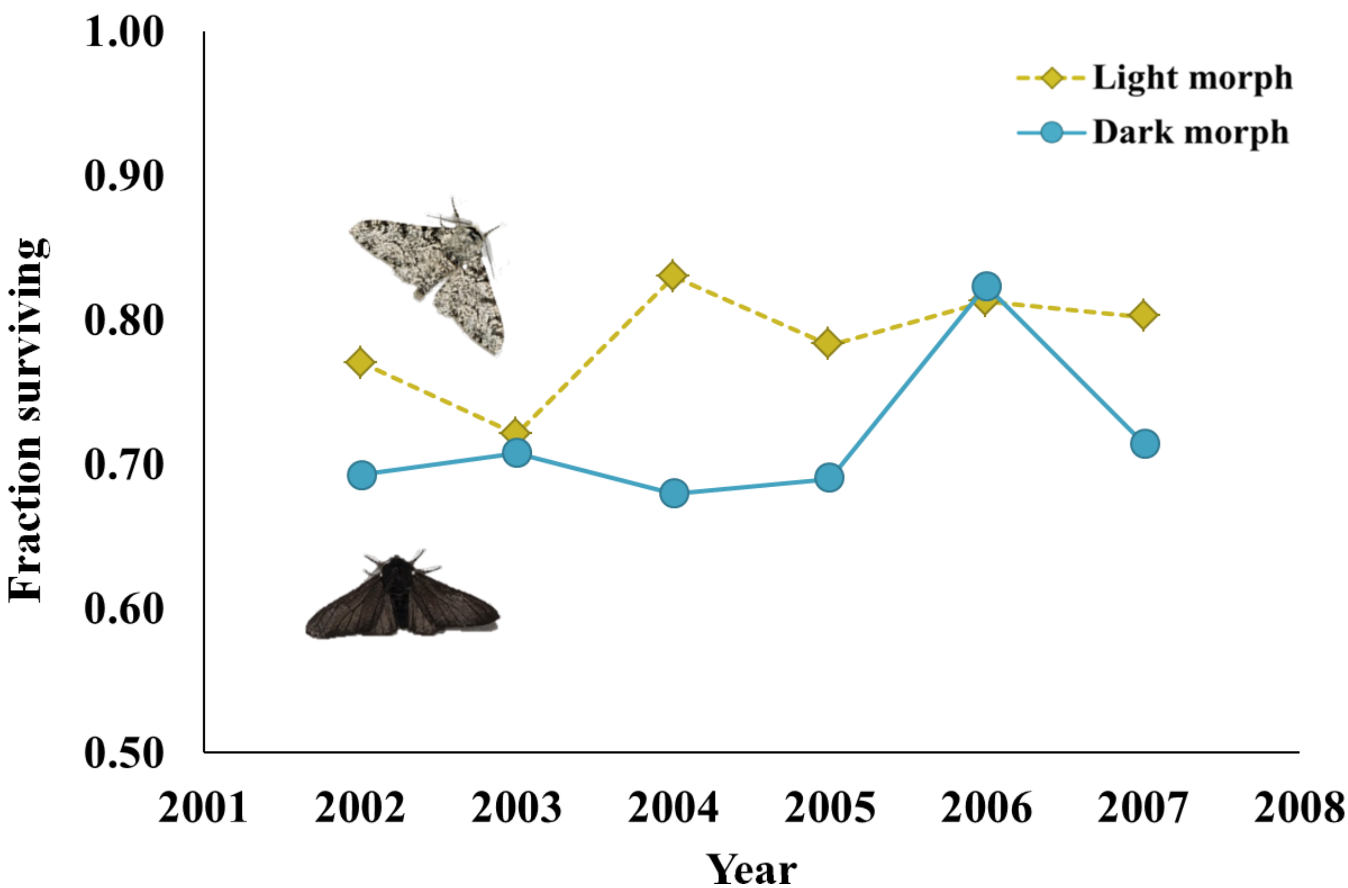


Figure 5. Proportions of light and dark morphs surviving over six years of predation experiments in an unpolluted forest. Data from Cook *et al.* (2012). Moth images by Chiswick Chap, CC BY-SA 2.5, <https://bit.ly/2H7Unt7> and <https://bit.ly/2LdirNU>.

Based on the results reported in Figure 5, Majerus was indeed able to demonstrate selective predation of dark morphs in an unpolluted forest. But to what degree do light morphs have a selective advantage in unpolluted forests? Your next task is to quantify the relative strengths of selection experienced by the light and dark morphs in an unpolluted environment using Majerus' data.

Quantifying Selection

One way to determine whether a phenotype is being selected for or against is to calculate the selection coefficient (s) for all phenotypes. (This value is usually calculated using genotype frequency data, but we will use Majerus' phenotype data instead.) The value of s is determined by first calculating the relative fitness (w) of each phenotype being considered—in this case, light and dark morphs. Using data from 2002 as an example, w_{dark} and w_{light} are calculated below. Note that w is always calculated with reference to the phenotype with the highest survival. After determining w, s may be calculated as: $s = 1 - w$.

$\text{survival}_{light} = (706 - 162) / 706 = 0.77$ $\text{survival}_{dark} = (101 - 31) / 101 = 0.69$

$w_{light} = 0.77/0.77 = 1.00$ $w_{dark} = 0.69 / 0.77 = 0.90$

$s = 1 - w_{light} = 1 - 1 = 0$ $s = 1 - w_{dark} = 1 - 0.9 = 0.1$

If $s = 0$ for a phenotype, that phenotype is not being selected against. If $s > 0$, the phenotype is being selected against, and the value of s can be used to describe the relative strength of selection against that phenotype. For example, because $s = 0.1$ for the dark morph in the above example, dark morphs have 10% more difficulty producing offspring than light morphs. Stated another way, dark morphs produce offspring at 90% the rate of light morphs.

Questions

1. Calculate the selection coefficient (s) for each morph in each year using the data below. Does either phenotype actually have a selective advantage in any year? If so, which phenotype? Are the results consistent across years?

Year	*Light morphs released*	*Light morphs eaten*	w_{light}	s_{light}	*Dark morphs released*	*Dark morphs eaten*	w_{dark}	s_{dark}
2002	706	162			101	31		
2003	731	204			82	24		
2004	751	128			53	17		
2005	763	166			58	18		

2. Using the value of s that you calculated for the year 2004, if 100 light moths produce 500 offspring, how many offspring would you expect 100 dark moths to produce?

3. It can be difficult to accept criticism, but criticism can often lead to improvement if it is taken seriously. Do you think Majerus' data are definitive, or are there still some assumptions that undermine the work? What do you think Kettlewell's reaction would have been on seeing Majerus' work and data?

Part V – Wing Length and Roadkill

Over 50 years after Kettlewell's original experiments, Majerus was able to provide definitive support for bird predation as the agent of selection in the case of Britain's peppered moth. Had Kettlewell more carefully considered the assumptions inherent in his data collection methods and addressed them experimentally, he could have more definitively reached the same conclusion.

Your final task is to consider the data presented in Figure 6, which describe a population of cliff swallows in Nebraska. These birds primarily nest under overhangs on highway bridges, overpasses, and culverts. After collecting data on this population for 30 years, Charles Brown and Mary Bomberger Brown were able to conclude that 1) the frequency of swallows collected as roadkill decreased over time and 2) birds collected as roadkill had longer wings than the average bird in the population.

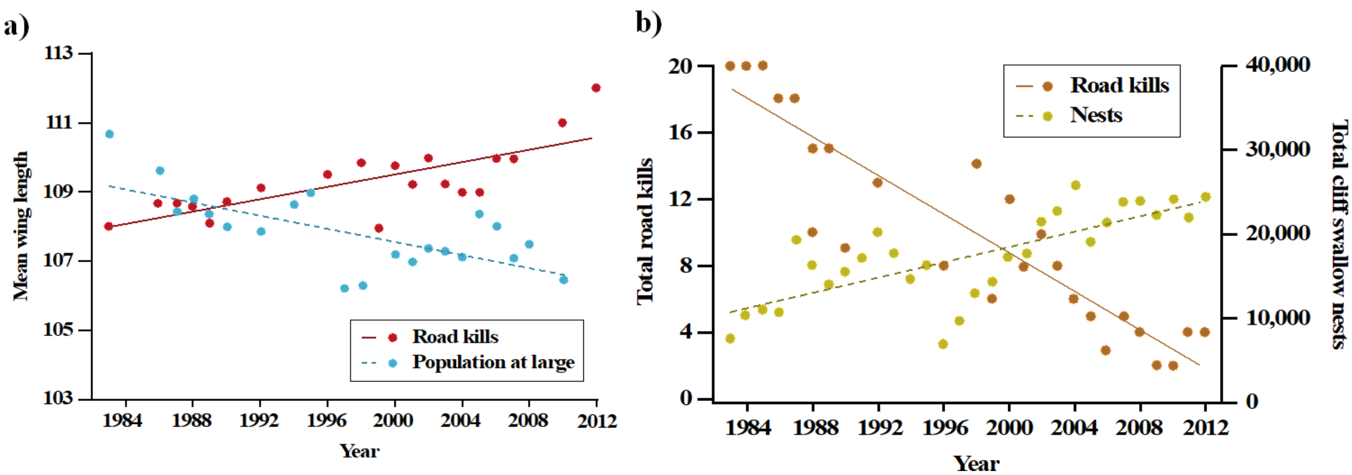


Figure 6. Data describing changes in a Nebraska cliff swallow population over time. (a) Mean wing length increases over time for birds collected as roadkill, but decreases for the population overall. (b) Nest number increases over time, indicating population growth, while the number of road kill events decreases over time. (Data from Brown & Brown, 2013).

Questions

1. Develop a hypothesis describing why wing length may be changing over time in this population, and specifically, for birds collected as roadkill.

2. Design an experimental approach to test your hypothesis. What are two assumptions that your methods do not directly address?

References

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Further Reading

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