

Las alas del Diablo: The Ecology, Evolution and Genetics of Capsaicin in Chilies

by

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By the end of the activity you should be able to:

- Read, describe and interpret graphical data, error bars and results of statistical tests.
- Identify the importance of experimental controls.
- Generate hypotheses for observations and identify when hypotheses can be discarded based on results of experiments.
- Draw conclusions based on experimental data supported with relevant and appropriate details.
- Using experimental evidence, determine if a trait could be considered an adaptation.
- Describe the ecological, evolutionary and genetic factors resulting in an adaptation.

Part I – Las alas del Diablo

[A couple of guys are sitting in a restaurant. A waitress comes to take their order.]

“Can I take your order?”

“Yeah, I’ll have the beef enchilada and a Coke.”

“And you?”

“What is this? “*Las alas del Diablo*?”

“Just like the menu says, devil wings. They are really spicy chicken wings. And you get six. The chef uses chilies she has been breeding for many years. She has developed some super hot varieties.”

“How hot?”

“Some people cry, most give up after the first two.”

“The menu says they are a hundred bucks; that can’t be right?”

“Well, sort of. If you finish all six in fifteen minutes they are free, and you get a milkshake, and your picture on the wall. If you don’t finish them, the contract says you donate a hundred bucks to the local food bank.”

“Contract?”

“The contract you sign if you order the wings. Don’t worry, you don’t need to sign in blood or anything. We use hot sauce.”

“Go on Phil, get the wings. You love hot stuff!”

“Ok, one order of *las alas del Diablo*. And blue-cheese dressing. That’ll cut the heat a bit.”

“You can have the wings but no dressings or drinks until the wings are done. The owner will stop by with the contract.”

Phil left the restaurant with his mouth burning and a hundred bucks poorer. He wondered why chilies were so hot. He knew chili plants make a chemical, capsaicin, which caused the burning sensation. But how could this benefit the plant?

His first thought was that capsaicin could be some kind of adaptation. He remembered that an adaptation is a type of trait that is inherited and increases the fitness of an individual with the trait compared with individuals without the trait in a particular environment. Fitness is a measure of the relative ability of individuals to produce offspring that survive and reproduce compared with other individuals in the population.

To be an adaptation, production of capsaicin would need to have a genetic basis so it could be inherited and increase the fitness of the plant relative to plants lacking capsaicin. Phil couldn't think of a reason to explain why making capsaicin would increase the fitness of a chili plant. Many plants produce fruits that turn bright red when they are ripe. The red color attracts birds, which eat the fruits. Birds often eat fruits whole, digest the fleshy part and poop out the seeds in a new location where they germinate and grow into new plants. It didn't make sense; how would a plant that made fruits to attract animals benefit from producing a chemical that might keep animals from eating the fruits?

Phil found part of the answer to his question in a study by Tewksbury and Nabhan (2001). The researchers wanted to determine if the capsaicin in chilies only deterred *some* types of animals from consuming the fruit.



Figure 1. Ripe and unripe fruit from a wild chili plant (*Capsicum annuum* var. *glabriusculum*). Credit: USDA, ARS plant germplasm collection, by R. Jarret, <<https://npgsweb.ars-grin.gov/gringlobal/accessiondetail.aspx?id=1047675>>.



Figure 2. Desert hackberry (*Celtis pallida*) showing fruit. Credit: William R. Carr, University of Texas at Austin, <<http://w3.biosci.utexas.edu/prc/DF/pages/CEPA8.html>>.

They made observations in the field to determine what animals ate wild chilies and other fruits. They chose a site in Arizona, which had wild chili plants (*Capsicum annuum* var. *glabriusculum*, Figure 1) and desert hackberry (*Celtis pallida*, Figure 2). The fruit of desert hackberries is similar in size to wild chilies. Wild chilies are long-lived shrubs, which belong to the same species (*C. annuum*) as domestic chilies and produce fruits containing capsaicin. The fruits of wild chilies are much smaller than those from cultivated plants.

To determine which animals ate chilies, the researchers observed plants at their study site during the day. Only birds, mostly curved billed thrashers (Figure 3), ate chilies during the day.



Figure 3. Curved billed thrasher (*Toxostoma curvirostre*). Credit: Alan D Wilson, CC BY-SA 3.0, <<http://carolinabirds.org/HTML/Mimids.htm#ThrasherCurvedBill>>.

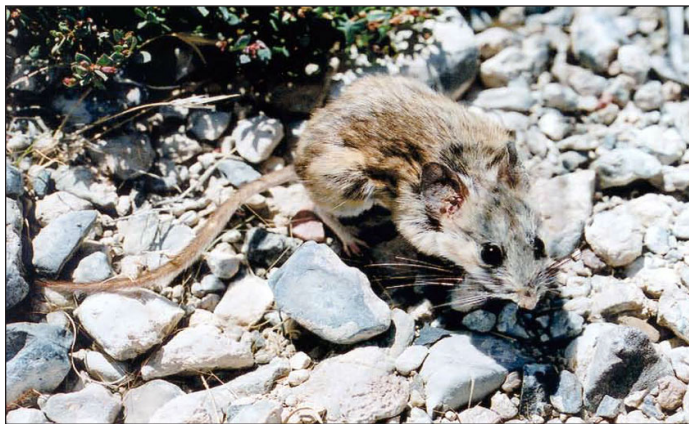


Figure 4. Cactus mouse (*Peromyscus eremicus*) in the Mojave Desert, Nevada. Credit: National Nuclear Security Administration / Nevada Site Office, p.d. <https://commons.wikimedia.org/wiki/File:Cactus_mouse_at_the_Nevada_Test_Site.jpg>



Figure 5. Packrat (*Neotoma lepida*). Credit: Jules Jardnier, CC BY-SA 4.0, <[https://commons.wikimedia.org/wiki/File:Desert_Packrat_\(Neotoma_lepida\)_eating_a_peanu_01.JPG](https://commons.wikimedia.org/wiki/File:Desert_Packrat_(Neotoma_lepida)_eating_a_peanu_01.JPG)>.

Rodents, like cactus mice (Figure 4) and packrats (Figure 5) could potentially consume chilies but they feed at night and were not observed feeding on any fruits.

To find out if rodents ate chilies, they performed a field experiment using two kinds of fruits, wild chilies and desert hackberry. Desert hackberry fruits don't contain capsaicin, are orange to red when ripe, are round and slightly smaller than wild chilies. Wild chili and desert hackberry fruits were placed in locations where birds and rodents could both access the fruits. During the day, both fruit types were removed with equal frequency; during the night, only hackberry fruits were removed from the sites. The researchers concluded that birds ate the wild chilies and rodents avoided the wild chilies.

It appears that something about the chilies deterred rodents but not birds from consuming the fruit. Deterring rodents from eating the fruit likely benefits the plant. When rodents eat fruits, they chew or gnaw both the fruit and seeds. This damages the seeds and prevents them from germinating (Tewksbury & Nabhan, 2001).

As Phil read about chilies he found the amount of variation in this species fascinating. Whether a plant produces capsaicin or not and the amount of capsaicin produced are genetically determined (Harvell & Bosland, 1997; Stewart *et al.*, 2005; Zewdie & Bosland 2000; Zewdie & Bosland 2000b). Green peppers (Figure 6), jalapeños and cayenne peppers (Figure 7) are all members of the same species, *C. annuum* (USDA, ARS). The amount of capsaicin differs a lot between a green pepper, which is not spicy at all, and jalapeños or cayenne peppers, which are very spicy.



Figure 6. Green pepper (*Capsicum annuum* var. *annuum*). Credit: B. Couch.



Figure 7. Cayenne peppers (*Capsicum annuum* var. *annuum*). Credit: B. Couch.

Part II – What Does Capsaicin Do?

Tewksbury and Nabhan (2001) are biologists who set out to test the hypothesis that capsaicin deters seed predators (rodents) from eating the fruits and seeds but not seed dispersers (birds). Deterring rodents should have a positive effect on plant fitness since rodents are thought to damage seeds. Seed dispersers should have a positive effect on plant fitness by dispersing seeds to new locations. There are two important predictions of this hypothesis: i) capsaicin deters rodents but not birds, ii) rodents reduce seed germination and birds have no effect or increase seed germination.

In their first lab experiment, the researchers tested the hypothesis that the presence of capsaicin in chili fruits deters rodents but not birds from consuming chilies. This was a simple food-choice experiment. They captured thrashers, packrats and cactus mice and brought them to the lab. In the experiment, individual animals were placed in cages and simultaneously given three different types of fruits: i) desert hackberries ii) mild chilies that lacked capsaicin (*Capsicum chacoense*) and iii) hot chilies (*C. annuum* var. *glabriusculum*). They then measured the percentage of each fruit that was eaten.

Each of the fruit types is considered a treatment; a combination of factors selected and regulated by the experimenters. The treatments in this experiment are summarized in Table 1 (next page). The hot chilies are the experimental treatment since they contain capsaicin, the feature expected to have an effect on fruit consumption. The other two treatments were controls, a positive control and a negative control. A control is an experimental group or treatment that serves as a reference to which a treatment group or treatment(s) can be compared. Hackberries were a positive control for feeding under laboratory conditions; even animals that had an aversion to chili-like fruits were likely to eat hackberries. The mild chilies were a negative control since they lacked capsaicin but were otherwise similar to chilies. If animals ate mild chilies, it would be possible to compare consumption of mild chilies to the consumption of hot chilies. A good control is as similar as possible to the treatment and should only differ in the factor of interest, in this case, capsaicin content.

For the negative control (mild chilies), fruits were chosen that were as similar as possible to the hot chilies but which lacked capsaicin. The mild chili was a mutant genotype of another species, *Capsicum chacoense* (Figure 8), which doesn't produce capsaicin. The hot and mild chilies are similar in size, color and nutritional content. Animals were presented with three fruit choices at the same time and the percentage of each fruit type consumed was recorded.

In the second lab experiment, the researchers tested the hypothesis that consumption of chilies by rodents but not birds reduces the survival of chili seeds. They fed mild chilies to thrashers, packrats and cactus mice and measured the percentage of seeds that germinated. They also collected seeds from ripe fruits and measured germination of seeds that had not been ingested by any animals. Since packrats and cactus mice won't eat hot chilies, they needed to use mild chilies, *Capsicum chacoense*, to measure the effect of fruit consumption on seed germination.

In addition to looking at the direct effect of consumption by birds on seeds, the researchers also assessed the effect of dispersal by thrashers on chili fitness. At their field site, the researchers found that dispersal of chili seeds by thrashers resulted in movement of seeds to locations that increased seedling survival and overall seedling fitness (Tewksbury & Nabhan, 2001).

As he continued his reading, Phil discovered that a gene, *Pun1*, was responsible for capsaicin production (Stewart *et al.*, 2005). The *Pun1* gene present in modern *Capsicum* species was present in the common ancestor of capsicum-containing species and inherited by all of the descendants of that ancestor.



Figure 8. Ripe and unripe from a wild chili plant (*Capsicum chacoense*) from the USDA, ARS plant germplasm collection. Credit: R. Jarret (USDA, ARS).

Some individuals of some species have mutations that disrupt the *Pun1* gene, resulting in mild chilies that don't produce capsaicin (Stewart *et al.*, 2005). Plants that don't produce capsaicin have two defective copies of the *Pun1* gene; the version of the gene for capsaicin production is dominant to the mutant versions of the gene.

Researchers have also discovered why birds and mammals respond differently to capsaicin. In mammals, capsaicin stimulates nerves responsible for sensing heat, which results in a burning sensation (Mason *et al.*, 1991; Jordt & Julius, 2002). The nerves from birds responsible for sensing heat are not stimulated by capsaicin; birds should not experience the burning sensation from consuming capsaicin (Mason *et al.*, 1991; Jordt & Julius, 2002).

Table 1. Summary of treatments in Tewksbury and Nabhan's 2001 feeding choice experiment.

| | Hackberry (<i>Celtis pallida</i>) | Mild Chili (<i>Capsicum chacoense</i>) | Hot Chili (<i>Capsicum annuum</i> var. <i>glabriusculum</i>) |
|---|---|---|--|
| <i>Treatment type:</i> | Positive control | Negative control | Experimental treatment |
| <i>Purpose of the treatment:</i> | Control for feeding under laboratory conditions and consumption of fruits other than chilies. Provides a comparison for consumption of chilies. | Control for consumption of chili-like fruits that lack capsaicin. Provides a comparison for consumption of hot chilies. | The treatment contains the factor, capsaicin that is expected to affect fruit consumption. |
| <i>Expected result if capsaicin deters seed predators but not dispersers:</i> | All animals should consume hackberries. | All animals should consume mild chilies. | Only birds should consume hot chilies. |

Questions

- In biology, what is fitness?
 - The ability of individuals to produce viable, fertile offspring compared with other individuals in a population.
 - The relative attractiveness of an individual in a population relative to other individuals in a population.
 - The relative physical strength of an individual in a population relative to other individuals in a population.
 - The individuals that survive the longest compared with other individuals in a population.
- What would you expect if the ability to produce capsaicin is genetic? Choose all that apply.
 - Some offspring of plants that produce capsaicin will also produce capsaicin.
 - It is not possible to predict if offspring of plants that produce capsaicin will also produce capsaicin.
 - Plants will only contain capsaicin if it is present in the soil and can be absorbed by the roots.
- In biology, what is an adaptation?
 - A trait with a genetic basis that increases the fitness of an individual with the trait in a particular environment, compared to individuals lacking the trait.
 - A characteristic of an organism that can change in response to the environment.
 - Any characteristic of an organism that increases the fitness of an individual in a particular environment, compared to individuals lacking the trait.
- Consumption of chili fruits by rodents would ...
 - Decrease the fitness of chili plants since gnawing damages the seeds.
 - Have no effect on the fitness of chili plants since the plant produces lots of seeds and fruits.
 - Increases the fitness of chili plants since seeds are dispersed to new locations.

5. How might dispersal of chili seeds by birds benefit a chili plant? Choose all that apply.
 - a. Seeds are transported to new locations so the offspring are not growing close to the parent and competing with it for light, water, space or minerals from soil.
 - b. Some seeds may be moved to locations with appropriate conditions for growth of seedlings.
 - c. Some seeds may be moved to locations that lack appropriate conditions for plant growth.
 - d. Seeds are widely dispersed so the offspring don't compete with each other for light, water, space or minerals from soil.
 - e. Seeds are damaged when the birds eat the fruits and fail to germinate.
6. Consumption of chili fruits by birds could ...
 - a. Increase the fitness of thrashers by providing nutrition, which can contribute to the increased production of offspring.
 - b. Have no effect on the fitness of thrashers since thrashers don't digest the seeds and the nutrition in the seeds would not contribute to increased production of offspring.
 - c. Reduce the fitness of thrashers since thrashers don't digest the seeds and the loss of nutrition in the seeds would reduce the production of offspring.
7. What is an experimental control?
 - a. An experimental group or treatment that serves as a reference to which a treatment group or treatment(s) can be compared.
 - b. A group that is not used in an experiment.
 - c. An experimental group or treatment that represents natural conditions.
 - d. An experimental group or treatment that lacks the factor of interest.
8. Does capsaicin production have a genetic basis?
 - a. Yes, capsaicin production is controlled by the *Hot2* gene.
 - b. Yes, capsaicin production is controlled by the *Pun1* gene.
 - c. No, if capsaicin is present in the soil it is taken up by the plant and concentrated in the fruit.
 - d. No, capsaicin is made by the plant not a gene.
9. What is (are) the control(s) in the food choice experiment performed by Tewksbury and Nabhan?
 - a. Hackberry
 - b. Mild chilies
 - c. Hot chilies
 - d. Hackberry and mild chilies
 - e. Mild chilies and hot chilies
10. Why is hackberry used as a control?
 - a. Hackberries are a control for fruit characteristics other than capsaicin (e.g., color, other chemicals).
 - b. Hackberries are a control for hunger or willingness to eat.
 - c. Hackberries are part of the natural habitat of cactus mice.
 - d. *a* and *b*.
 - e. *a*, *b* and *c*.
11. What criteria must be met for a trait to be considered an adaptation?
 - a. Individuals with the trait generally have more offspring than individuals without the trait.
 - b. The trait changes due to the environment (e.g., animals growing a thick coat in winter).
 - c. The trait can be passed on to an organism's offspring.
 - d. *a* and *c*.
 - e. *a*, *b* and *c*.
12. Refer to the results of Tewksbury and Nabhan's field experiments and observations; do *not* refer to the lab experiments from Part II. Provide at least two reasonable, alternative, biological explanations for why rodents did not eat chilies.

References

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Additional Reading

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