How-To-Do-It

Studying the Genetics of Behavior & Evolution
By Adaptation & Natural Selection

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Inheritance mechanisms can be studied in the classroom using phenotypic markers from a number of sexually reproducing organisms. Morphological traits, distinguished by size, shape or color, are generally unambiguous and therefore most amenable to classroom study. Far fewer behavioral traits following Mendelian inheritance patterns have been discovered, and these may not be suited for study in a high school or undergraduate biology laboratory.

Recently, Silverman and Bieman (1993) and Silverman and Ross (1994) discovered populations of household cockroaches, Blattella germanica, that survived treatment with a once very effective insecticidal bait formulation. Survival was not in response to individuals predisposed to resist the toxic effects of the insecticide, nor did these isolated populations avoid the insecticide: the two most likely explanations for product failure. Surprisingly the surviving individuals exhibited a strong aversion to glucose, an energy source added to stimulate ingestion of the toxicant. Rather than being learned, glucose-aversion is a semi-dominant-autosomal trait, apparently controlled by a single major gene. Subsequently, Ross and Silverman (1995a & b) mapped this behavior to chromosome 9. These were the first reports of aversion to a typically phagostimulatory sugar and nutrient avoidance as a resistance mechanism.

The following exercise was designed to give the student an appreciation for the genetic basis of behavior. Furthermore, like industrial melanism and antibiotic and insecticide resistance, glucose-aversion serves as an example of evolution by mutation and accelerated natural selection, thereby revealing one of the many ways that organisms adapt to human interference.

The experiments use cockroaches, which despite their unsavory reputation, are ideal subjects for laboratory investigation at all education levels (Bell 1981). They are readily available, inexpensive, hardy, easy to maintain and culture, and very suitable for demonstrating a variety of biological principles at the level of the cell, organism and population.

Materials

- Plastic or glass petri dish bottom (90 mm diam.)
- Filter paper and laboratory wipe or tissue paper
- Petrolatum and mineral oil mix (1:1)
- Glucose
- Amaranth (Sigma)
- Hypodermic syringe (5 ml)
- Boric acid
- Dog chow (Purina®)
- Cotton stoppered water vial
- Small (ca. 1 cm diam.) vial cap
- Tygon tubing (20 mm x 3 mm diam.)
- Wild-type (glu+/glu+), glucose-averse (glu+/glu) and heterozygous (glu+/glu & glu/gl+ +) Blattella germanica nymphs (from author)
- Reference card with dots of various intensity reflecting glucose consumption by genotype (from author)

The filter paper should be cut to fit snugly in the bottom of the dish with the wipe or tissue placed tightly on top of the filter paper. Nymphal cockroaches (ca. 3 mm in length) will be placed on the wipe and should not escape this surface. Apply the petrolatum/mineral oil mixture as a thin film to the inside vertical surface of the dish to prevent insect escape. The dish can be placed in a larger lubricated container as an additional precaution. Prepare a single aqueous solution of 2 molar glucose and 8 millimolar amaranth. Prepare a 5% aqueous boric acid and 1 molar glucose solution. Cut the barrel (cross section) of the hypodermic syringe so that there are no gaps when pressed against a horizontal surface. Remove the rubber gasket from the plunger of the syringe.

Procedure

Determining Dominance & Sex-Linkage

1. Place glu+/glu+, glu/glue, male glu+/female glu, and male/female glu+ offspring of the provided gravid females into one petri dish (prepared as above) per genotype. Since the relative amounts of glucose ingested will be compared, the nymphs should be approximately the same size, not more than 10 days old.
2. Deprive the nymphs of food and water for two days. This period of deprivation intensifies the reaction to glucose. Nymphal B. germanica will survive ca. five days without food and water.
3. Place a length of tygon tubing, filled with the glucose/amaranth solution, into each petri dish. Carefully dry the outside of the filled tube before placing it on the paper. Leave in place for 15 minutes, then remove. You will notice

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4. Lift the plunger of the modified hypodermic syringe slightly to create a space to entrap individual nymphs. Place the syringe above the nymph; then depress the plunger, crushing the nymph. After all the nymphs have been crushed, remove the tissue paper with the cockroach remains. The bottom layer of filter paper will contain red spots of varying intensity which roughly approximate the quantity of glucose imbibed.

5. Compare spots recovered from nymphs with reference card.

The vast majority of the darkest spots belong to the glu+/glu+ insects, indicating no aversion to glucose. Some glu+/glu+ individuals may not encounter the solution within the 15-minute period. This is not aversion, just limited foraging activity. All the glucose-averse (glu/glu) nymphs will reveal faint or no spots exemplifying the intensity of aversion to this sugar despite two days of food deprivation. The residues from both heterozygous reciprocal crosses will be very faint, indicating that glucose aversion behavior is nearly completely dominant, and it is not sex linked. An example of the dye intensities produced following ingestion by these four genotypes appears in Table 1.

**Determining Monogenic Versus Polygenic Inheritance**

This experiment is conducted as above using offspring from a cross between female glu+/glu+ and male glu/glu+ individuals. Students can conduct the crosses by sparing some nymphs from the above genotypes and rearing them to the adult stage; however, it would take ca. two months to obtain the needed offspring. Alternatively, gravid females containing glu+/glu+ and glu/glu+ progeny can be provided by the author. The distribution of dye intensities from the crushed nymphs will depend on whether the glucose aversion trait is controlled by one or more genes. If controlled by one gene, then two expression patterns will be observed, one for glu/glu+ and one for glu+/glu+. If the behavior is quantitative or polygenic, a normal distribution of dye intensities will be observed. Glucose aversion is controlled by one major gene. An example of the distribution of dye intensities one could expect from this experiment appears in Table 2 along with bimodal and normal frequency curves for the two modes of inheritance (Figure 1).

Monogenic vs. polygenic inheritance can be determined mathematically using the chi-square ($\chi^2$) goodness-of-fit test (Gardner & Snustad 1984). The formula to determine $\chi^2$ is:

$$\chi^2 = \frac{(O_1 - E_1)^2}{E_1} + \frac{(O_2 - E_2)^2}{E_2}$$

where $O_1$ is the experimentally observed number for the first class (dye intensity), and $E_1$ is the expected number for the same class derived from the ratio (i.e. 1:1 or 3:1 depending on inheritance mechanism); $O_2$ is the observed for the second class and $E_2$ the expected.

According to the distribution of backcross offspring (Table 2, Row 3) two main dye intensity categories can be created, columns 1 & 2 and columns 4 & 5. The observed frequency for the first category is 44 (34 + 10) and 36 (10 + 26) for the second category. For monogenic inheritance, we expect a 1:1 ratio: one-half of the offspring will be “glucose-loving” (glu+/glu+); the other half will be heterozygous (glu/ glu+) and avoid glucose. The total sample size is 80; therefore, we expect 40 for $E_1$ and 40 for $E_2$. Substituting into the above formula:

$$\chi^2 = \frac{(44 - 40)^2}{40} + \frac{(36 - 40)^2}{40}$$

$\chi^2 = 0.8$, which on a table of $\chi^2$ values falls between 0.45 and 1.64. The
The probability of obtaining a deviation as great as, or greater than, \( \chi^2 = 0.8 \) is between 0.2 and 0.5. Such a deviation could be explained by chance, and therefore the data do not deviate significantly from a 1:1 ratio, indicating that the trait is most likely controlled by a single major gene.

**Evolution by Adaptation & Natural Selection**

The frequency of genes controlling specific traits can be increased by imposing a selective force to remove individuals that lack these genes from the population. Cockroaches with the glucose-aversion adaptation (gene) were scarce in human dwellings until exposure to glucose-toxic mixtures (baits) skewed populations from "glucose-loving" to glucose-averse within five years. The following exercise demonstrates the selection process involved.

1. Place various ratios of glu/glul+/glu+ nymphs in petri dishes prepared as described above. Possible ratios include 2:98, 20:80, 50:50.
2. Deprive of food and water for two days, then place a vial cap with boric acid/glucose solution with cockroaches for 1–2 hours. Remove the cap, then add a dog chow nugget and a cotton stoppered water vial.
3. Boric acid should exert its toxic effect within five days. Count and record the number of dead and live nymphs.

The surviving nymphs will be those that avoided the boric acid/glucose solution. These glucose avoiders now make up the entire cockroach population by eliminating the non-glucose avoiders. Although the frequency of the glucose aversion gene in this experiment is considerably higher than would be expected within the insects' natural habitat, the same human-induced evolutionary process occurs in nature, albeit more slowly.

For anyone that has battled insects in their homes, this exercise should provide the student with an appreciation for how evolution can directly affect their lives. A once-effective cockroach bait lost its effectiveness in some locations, not because the bait changed, but because the cockroach population changed to favor those individuals that had an inherited aversion to the food material, glucose. Removal of glucose from the bait restored its effectiveness.

**Summary**

The experimental protocols described in this article allow for hands-on experience in investigating two important concepts: 1) Behavior has a genetic component, and 2) human activity can accelerate the evolution of certain traits in other organisms by providing a selective force. The techniques and materials employed are simple, inexpensive, and readily adaptable to high school or college biology or genetics laboratories.

**References**

