

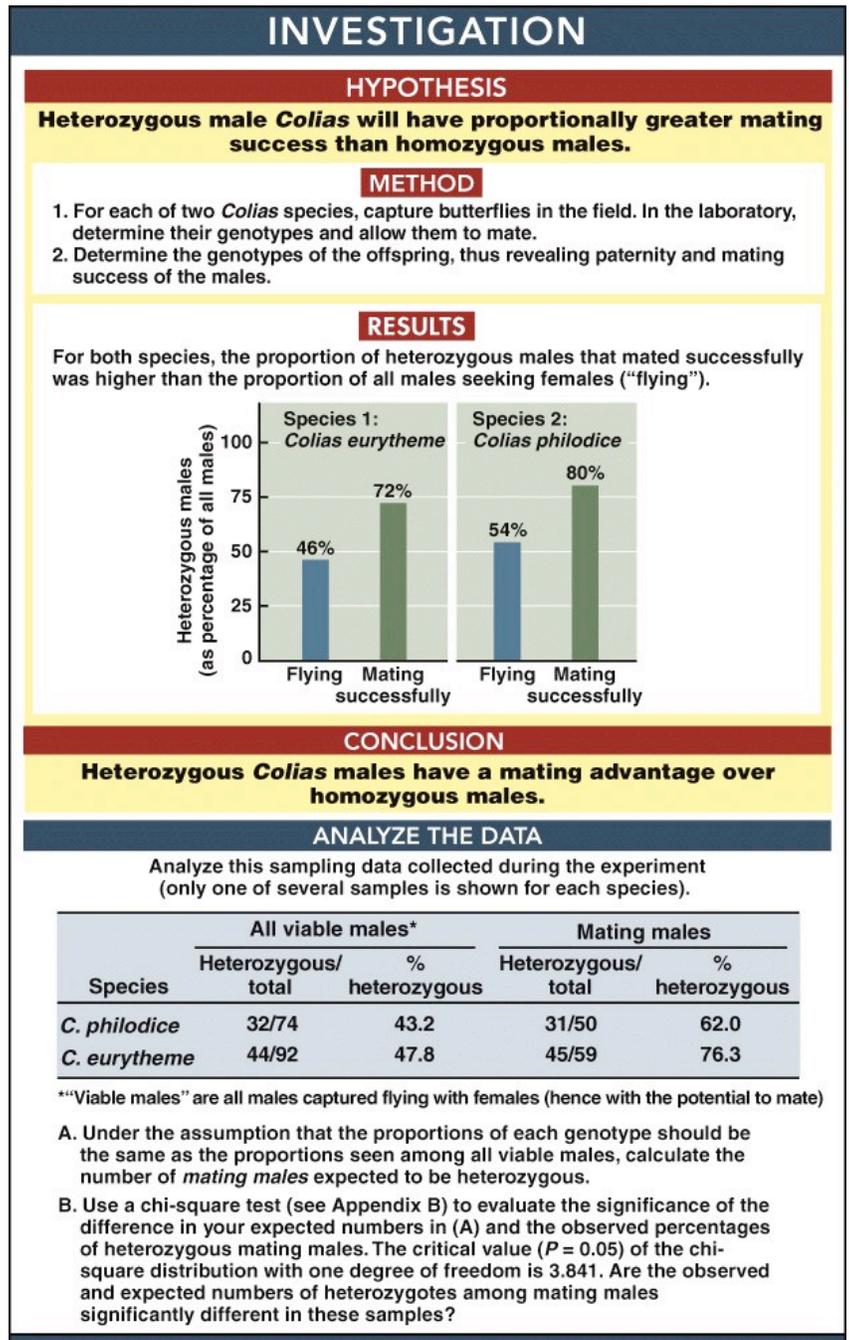
Working With Data from *Principles of Life* by Hillis

Determining the Paternity of a Butterfly Larvae

Introduction

Multiallelic polymorphisms of the enzymes at the phosphoglucose isomerase (PGI) locus are common in *Colias* butterflies in nature. Previous studies demonstrated that flight ability of *Colias* butterflies depends on the presence of particular PGI alleles and that heterozygotes can fly farther under a broader range of temperatures than homozygotes can. These findings led Ward Watt, Patrick Carter, and Sally Blower to hypothesize that heterozygous male *Colias* butterflies would have better mating success in nature than homozygous males. To test this prediction, they captured butterflies in the wild, determined their genotypes, allowed females to lay eggs in captivity, and determined the genotypes of their offspring. These data allowed them to determine the genotype of the fathers and, therefore, measure mating success rate. They found that in two different species of butterflies, *Colias eurytheme* and *Colias philodice*, the heterozygous males represented only 46 percent and 54 percent, respectively, of the flying population. In contrast, these same males accounted for 72 percent and 80 percent, respectively, of successful matings. These data are consistent with the hypothesis that heterozygous males have a mating advantage over homozygous males.

An additional example of a mating advantage attributable to genotype is found in *Drosophila melanogaster*. J. Rendel (1951) and M. Jacobs (1960) independently described the influence of light on mating behavior of *ebony* and *non-ebony* *Drosophila melanogaster*. Interestingly, both researchers found that *ebony* flies showed greater sexual activity in the dark as compared to the light, while *non-ebony* flies showed greater sexual activity in the light as compared to the dark. In 1978, C. Kyriacou and colleagues demonstrated that flies heterozygous for *ebony* appear to have a selective advantage over both homozygotes. The *ebony* gene encodes the enzyme N-β-alanyldopamine (NBAD) synthetase that



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converts dopamine to NBAD, which is subsequently oxidized to produce a yellowish pigment. In *ebony* mutants, the body color of the fly varies from black to slightly darker than wild-type, depending on allele.

Original Papers

Jacobs, M. 1960. Influence of light on mating of *Drosophila melanogaster*. *Ecology* 41: 182–188.
<http://www.jstor.org/stable/1931952>

Kyriacou, C. P., B. Burnet, and K. Connolly. 1978. The behavioural basis of overdominance in competitive mating success at the *ebony* locus of *Drosophila melanogaster*. *Animal Behaviour* 26: 1195–1206.

http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6W9W-4F1RW93-C5&_user=108429&_rdoc=1&_fmt=&_orig=search&_sort=d&_docanchor=&_view=c&_acct=C000059713&_version=1&_urlVersion=0&_userid=108429&md5=de0812e4a29b7b3269b0a49ca1efcca2

Rendel, J. M. 1951. Mating of *ebony*, vestigial and wild-type *Drosophila melanogaster* in light and dark. *Evolution* 5: 226–230.

<http://www.jstor.org/stable/2405462>

Watt, W. B., P. A. Carter, and S. M. Blower. 1985. Adaptation at specific loci. IV. Differential mating success among glycolytic allozyme genotypes of *Colias* butterflies. *Genetics* 109: 157–175.

<http://www.genetics.org/cgi/reprint/109/1/157>

Links

(For additional links on this topic, refer to the Chapter 15 Investigation Links.)

PubMedCentral: Genetics: The Influence of Light on Gene Frequency Changes in Laboratory Populations of Ebony and Non-Ebony Drosophila Melanogaster

<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1210260>

University of Virginia: Evolution Lab with Drosophila (pdf)

<http://www.faculty.virginia.edu/evolutionlabs/DrosophilaEvoBioscenev28-2p3-6.pdf>

Analyze the Data

Colias philodice and *C. eurytheme* are very closely related species; they occasionally hybridize where their ranges overlap. Experiments on *C. philodice* were carried out at Gunnison, Colorado (elevation 2350 m). Experiments on *C. eurytheme* were carried out at Tracy, California (elevation 30 m), far removed from where their ranges overlap.

The researchers were aided by several important facts. First, even though a female *Colias* may mate with more than one male, sperm precedence is complete. That is, a female's most recent mate fertilizes all of her subsequent eggs. Thus, eggs laid by a female during a given period have only one father. Second, females readily lay eggs in captivity and the larvae are easy to rear on an artificial diet. Third, the genotype of the mothers and fathers can be determined by electrophoresis of the larvae.

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PGI influences the thermal dependence of flight because one of the steps in the metabolic pathway that butterflies use to fuel the ATP in their flight muscles is governed by PGI. Heterozygotes have a superior ability to sustain flight, especially outside the optimal body temperature of 35°C–39°C. Four alleles at the PGI locus that are frequent in the wild were analyzed in these experiments.

Temperatures during the *Colias* mating season fluctuate widely during the day. Most of the flight time of *Colias* males is spent patrolling the tops of the vegetation searching for newly receptive females. Most females emerge from their pupae in the cool hours early in the day when heterozygotes should have a strong advantage. The males find females and court them; the females choose.

Question 1

The investigators subjected enough larvae from each batch of eggs to judge the genotype of the father with 99 percent certainty. How many larvae did they need to measure to achieve that level of certainty? (Hint: If the female is a homozygote, say, *ii*, the number needed is small. However, if a female is a heterozygote, say, with genotype *ij* and only *ii* and *ij* progeny are found in her brood, more larvae need to be genotyped.) In this particular case, the father can be only *ii* or *ij*. If he were *ij*, the probability that any one offspring is not *jj* = 0.75, so the chance of getting only *ii* and *ij* among *n* offspring is 0.75^n . What *n* is required to reduce the probability of error in determining his genotype to 0.01?

Table 1 Genotype frequency comparisons between sampled viable males and males successfully mating.

Comparison	Sample (Date)	Males Viable*		Males Mating	
		Number	%	Number	%
Heterozygotes with Homozygotes	<i>C. philodice</i> (8/8/1983)	32/74	43.2	31/50	62.0
	<i>C. eurytheme</i> (9/24/1983)	38/78	48.7	58/80	72.5
	<i>C. eurytheme</i> (11/3/1985)	44/92	47.8	45/59	76.3

*Males Viable = Males caught flying with females in the wild.

Question 2 (from textbook Figure 15.19)

Analyze this sampling data collected during the experiment (only one of several samples is shown for each species).

Species	All viable males*		Mating males	
	Heterozygous/total	% heterozygous	Heterozygous/total	% heterozygous
<i>C. philodice</i>	32/74	43.2	31/50	62.0
<i>C. eurytheme</i>	44/92	47.8	45/59	76.3

*"Viable males" are all males captured flying with females (hence with the potential to mate)

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A. Under the assumption that the proportions of each genotype should be the same as the proportions seen among all viable males, calculate the number of *mating* males expected to be heterozygous.

B. Use a chi-square test (see Appendix B) to evaluate the significance of the difference in your expected numbers in (A) and the observed percentages of heterozygous mating males. The critical value ($P = 0.05$) of the chisquare distribution with one degree of freedom is 3.841. Are the observed and expected numbers of heterozygotes among mating males significantly different in these samples?

Question 3

In Question 2 above, you analyzed the frequencies of heterozygous males among all viable males and mating males. In all three tests, heterozygote males were over-represented among the males that actually mated. But, do these results show that heterozygote males achieved their success because they could sustain flight longer than homozygote males? What other hypothesis could explain these results? (Propose some alternatives before you go to the next question.)

Question 4

A possible alternative hypothesis to explain why heterozygous males disproportionately father offspring is that females that mate with those males are more likely to lay eggs than females that mate with homozygous males. The investigators rejected that hypothesis because nearly all females laid fertilized eggs in captivity. Another alternative is that fitness differences were not due to alleles at the PGI locus but were due to genes at other loci on the same chromosome. A third possibility is that heterozygote males, being more vigorous flyers, are more difficult to capture. If so, the investigators might have underestimated the frequency of heterozygotes in the wild. The investigators tested and rejected these alternatives. What data could they have used to accomplish that task?