

Phenocopy Experiment (1/2001)

Exposure to certain environmental conditions induces phenotypic changes which resemble those caused by known mutant genes (phenocopies). In some cases, the phenocopying agent effects the same developmental pathway as does the mutant gene itself. Certain genotypes are more susceptible to phenocopy induction than are other genotypes. We will examine the phenocopying effect of sodium metaborate upon normal flies, and upon flies which are heterozygous for one of two mutant alleles: eyeless (*ey*) or Lobe (*L*). Each of these two mutations (*ey/ey* or *L/-*) produce a reduction in the size of the eye in normal media. We will use the chi-square contingency test to compare the response of normal flies (+/+) and heterozygotes for each of these mutations (+/*ey*, or *L/+*) to the phenocopying agent.

Media: (C) control - The usual Carolina Instant *Drosophila* media.

(E) experimental - Instant *Drosophila* media made up with a 0.07% solution of sodium metaborate (0.7g sodium metaborate in 1 liter of distilled water)

Crosses: (1) 6 wild type pairs (virgin ♀ are not necessary).

(2) 6 virgin eyeless ♀ x 6-8 wild type ♂ (or the reciprocal cross).

(3) 6 virgin Lobe ♀ x 6-8 wild type ♂ (or the reciprocal cross).

Cross	Media	
	C	E
1	5 vials	5 vials
2	5 vials	5 vials
3	5 vials	5 vials

Note that each vial (except +/+ ♀) will have 6 of the appropriate virgin females in it. Thus you will need to collect  $4 \times 5 \times 6 = 120$  virgin females for this experiment.

Remove the parents after 5-7 days. Score the  $F_1$  of each of the groups for 7 days after first possible emergence

Analyses: Make selected comparisons using contingency chi-square ( $\chi^2$ ) tests. This test is called a test for independence, and tests the similarity of two populations for the presence or absence of some trait. Separately test the control vs. each treatment.

$$H_0 : x = y \quad H_1 : x \neq y$$

Trait	Treatment		$\Sigma$
	control	experimental	
reduced eye	a	b	a+b
normal eye	c	d	c+d
$\Sigma$	a+c	b+d	a+b+c+d

$$\chi^2 = \frac{(ad-bc)^2 n}{(a+c)(b+d)(a+b)(c+d)} \quad df = 1$$

Some tabular Chi-square values with 1 degree of freedom are shown below.

$$\chi^2_{.05} = 3.841 \quad \chi^2_{.01} = 6.635 \quad \chi^2_{.005} = 7.879 \quad \chi^2_{.001} = 10.828$$

If your observed  $\chi^2$  value is greater than 3.841, reject  $H_0$  which states that there is no significant difference between populations. Therefore accept  $H_1$ .

For example, suppose that the results of a comparison in which the flies were all raised in sodium metaborate media are:

Trait	Treatment		$\Sigma$
	+/+	+/ey	
reduced eye	60	90	150
normal eye	140	110	250
	200	200	400

$$\chi^2 = \frac{(/60 \times 110 - 90 \times 140/)^2}{(200)(200)(150)(250)} \cdot 400 = 9.60 \quad P < .005$$

This indicates that the frequency of the phenocopy is dependent upon the genotype of the flies. There are significantly more +/ey flies (45%) than +/+ flies (30%) with reduced eyes.

Make the following comparisons:

- 1) +/+ in normal media v.s. +/+ in sodium metaborate media.
- 2) +/ey in normal media v.s. +/ey in sodium metaborate media.
- 3) L/+ in normal media v.s. L/+ in sodium metaborate media.
- 4) +/+ v.s. +/ey in normal media.
- 5) +/+ v.s. +/ey in sodium metaborate media.
- 6) +/+ v.s. L/+ in normal media.
- 7) +/+ v.s. L/+ in sodium metaborate media.

Write and submit a report on your work. Show all calculations.