

Laboratory 7: Genetics of *Drosophila*

- *conduct a genetics experiment for a number of generations
- *compare predicted results with actual results
- *explain the importance of chi-square analysis
- *design genetic crosses in an experiment to illustrate independent assortment and sex-linkage
- *discuss the life cycle of the fruit fly, recognize the sex of fruit flies, and recognize several classic types of mutations

Drosophila melanogaster (literally, "dew-lover, black-bellied") is a "perfect" lab animal on which to perform experimental genetic crosses. It is small, requires minimal upkeep and food, is quiet and odorless, reproduces very quickly (new generation in two weeks), and has many easily observable phenotypes resulting from different alleles.

A fly house is made (blue media mixed in a plastic vial, yeast added to provide food for maggots, netting inserted as structure for pupation) and pure-breeding (homozygous) parents are introduced.....

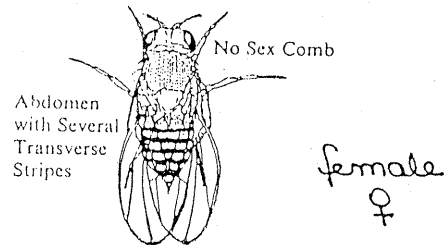
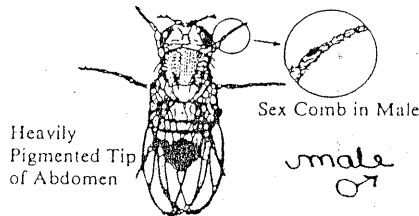
P_1 = 3 vestigial-winged males X 3 normal-winged females

These flies mate, are removed after 48 hours and maggots are observed 5 days later.....pupae are observed after 10 days. When the F_1 generation begins to hatch out, several are observed, and all exhibit normal wings.

Therefore, you can deduce that normal is dominant over vestigial!

A new fly house is prepared and 3 male and 3 female F_1 s are added. After several days, they are removed....at the end of two weeks, the F_2 generation begins to hatch out.

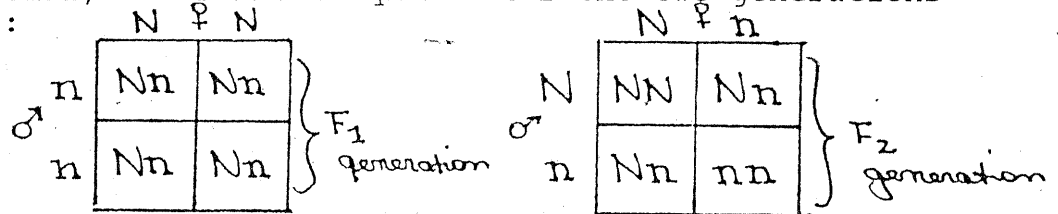
To make calculations easy, 100 F_2 flies are counted. For ease of observation, foam plugs are quickly removed and vials placed upside down on containers of ice: the flies fall to the surface and become "chilled" which renders them immobile and easy to sex (see below) and count.



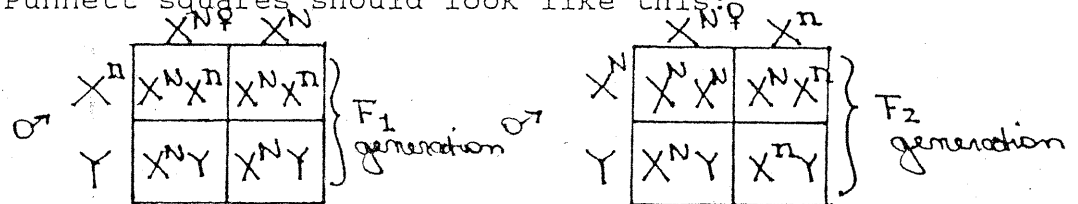
At the end of the count, 43 normal females
33 normal males
8 vestigial females
16 vestigial males are observed.

Remember, there are two possibilities: this trait may be autosomal or sex-linked. If autosomal, the Punnett squares for the two generations should look like this:

N = normal
n = vestigial



If sex-linked, the Punnett squares should look like this:



Observing the results and comparing, it seems as if the trait must be autosomal. Therefore, the expected results should have been 3/8 normal males, 3/8 normal females, 1/8 vestigial males, 1/8 vestigial females: using this information, a Chi-square analysis can be run to determine how close the actual results are to the expected!

Degrees of Freedom	P = .99	.95	.80	.50	.20	.05	.01
1	.000157	.00393	.0642	.455	1.642	3.841	6.635
2	.020	.103	.446	1.386	3.219	5.991	9.210
3	.115	.352	1.005	2.366	4.642	7.815	11.345
4	.297	.711	1.649	3.357	5.989	9.488	13.277
5	.554	1.145	2.343	4.351	7.289	11.070	15.086
6	.872	1.635	3.070	5.348	8.558	12.592	16.812
7	1.239	2.167	3.822	6.346	9.803	14.067	18.475
8	1.646	2.733	4.594	7.344	11.030	15.507	20.090
9	2.088	3.325	5.380	8.343	12.242	16.919	21.666
10	2.558	3.940	6.179	9.342	13.442	18.307	23.209
15	5.229	7.261	10.307	14.339	19.311	24.996	30.578
20	8.260	10.851	14.578	19.337	25.038	31.410	37.566
25	11.524	14.611	18.940	24.337	30.675	37.652	44.314
30	14.953	18.493	23.364	29.336	36.250	43.773	50.892

$$\chi^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

$$\chi^2 = \frac{(43-37.5)^2}{37.5} + \frac{(33-37.5)^2}{37.5} + \frac{(8-12.5)^2}{12.5} + \frac{(16-12.5)^2}{12.5} =$$

$$1.13 + 0.54 + 1.62 + 0.91$$

★ 4.27 at 3 degrees of freedom

the level of significance is close to .2 / acceptable!

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Question 1

In fruit flies, the phenotype for eye color is determined by a certain locus. *E* indicates the dominant allele and *e* indicates the recessive allele. The cross between a male wild-type fruit fly and a female white-eyed fruit fly produced the following offspring.

	Wild-type Male	Wild-type Female	White-eyed Male	White-eyed Female	Brown-eyed Female
F1	0	45	55	0	1

The wild-type and white-eyed individuals from the F1 generation were then crossed to produce the following offspring.

F2	23	31	22	24	0
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- Determine the genotypes of the original parents (P generation) and explain your reasoning. You may use Punnett squares to enhance your description, but the results from the Punnett squares must be discussed in your answer.
- Use a Chi-squared test on the F2 generation data to analyze your prediction of the parental genotypes. Show all your work and explain the importance of your final answer.
- The brown-eyed female in the F1 generation resulted from a mutational change. Explain what a mutation is, and discuss two types of mutations that might have produced the brown-eyed female in the F1 generation.

Critical Values of the Chi-Squared Distribution

Probability (p)	Degrees of Freedom (df)				
	1	2	3	4	5
0.05	3.84	5.99	7.82	9.49	11.1

The formula for Chi-squared is:

$$\chi^2 = \sum \left[\frac{(o - e)^2}{e} \right]$$

where *o* = **observed** number of individuals

e = **expected** number of individuals

Σ = the **sum of the values** (in this case, the differences, squared, divided by the number expected)

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Question 1 (continued)

a) Maximum 4 points

- 1 pt Genotypes of the parents (words or symbols) $X^E Y$ (or $X^+ Y$) and $X^e X^e$
- 1 pt Discuss/show how these resulted in this F1 (may be annotated Punnett)
- 1 pt Explain that it is a sex-linked (X-linked) gene (not just the word)
- 1 pt How you know which type is dominant
- 1 pt F2 results (may be annotated Punnett square)

b) Maximum 4 points

- 1 pt Correct F2 hypothesis (1:1:1:1; or 25/genotype)
- 1 pt Show work (components): $\frac{o}{e} \frac{o-e}{(o-e)^2} \frac{(o-e)^2}{e}$
(or correct numbers $4/25 + 36/25 + 1/25 + 9/25 = 50/25 = 2$; or at least the last term)
- 1 pt Sum: correct chi-square result ~ 2.0 or 1.85
- 1 pt degrees of freedom = 3 (critical value is 7.82)
- 1 pt correct interpretation of chi-square in terms of p
p = probability that the difference between the observed and the expected value is due to chance alone.
This p value shows we accept our hypothesis.
The null hypothesis is supported in this case.
(alternative: 2 X^2 tests of white vs. red males and white vs. red females)

c) Maximum 4 points

- 1 pt Explain what a mutation is: (heritable) change in the DNA (code)
- 1-2 pts Discuss 2 types of mutations
May be: Point mutation, frameshift (deletion/duplication), insertion, transposition, break, inversion
within gene, base substitution, nonsense/stop, missense)
May NOT be: chromosomal aberration, nondisjunction, silent/neutral, transcription or translation
or processing error
- 1 pt Molecular or biochemical elaboration beyond the explanation required

other factor.

c.) A mutation is a change in an organism's genetic code which, if not discovered by proofreading mechanisms, creates abnormal phenotypic ^{traits} ~~events~~ for the to appear in the organism. There are a number of different kinds of mutations, ^{any} all of which may have occurred to create the brown-eyed female. A ^{extra} nucleotide may have been added to the fly's genome, in which is referred to as a ^{insertion} ~~frameshift~~ mutation (a deletion, in which a nucleotide was removed, may also have occurred). The insertion would also cause a frameshift mutation in which the three-base "codon" reading frame was all altered ^{to} due to the addition of the extra nucleotide. Nucleotides within the fly's genome could also ^{their} ~~possibly~~ have inverted positions, in which is referred to as an inversion mutation.

1pt

1pt

1pt

1pt
(+)

GO ON TO THE NEXT PAGE.

Critical Values of the Chi-Squared Distribution

Score = 10

Probability (p)	Degrees of Freedom (df)				
	1	2	3	4	5
0.05	3.84	5.99	7.82	9.49	11.1

The formula for Chi-squared is:

$$\chi^2 = \sum \left[\frac{(o-e)^2}{e} \right]$$

where o = observed number of individuals

e = expected number of individuals

Σ = the sum of the values (in this case, the differences, squared, divided by the number expected)

- a) The original genotypes of the parents were $X^E X^E$ (female) and $X^E Y$ (male). When these two genotypes are crossed using a punnett square, the following results are achieved:

	X^E	X^E
X^E	$X^E X^E$	$X^E X^E$
Y	$X^E Y$	$X^E Y$

This yields a ratio of 2 wild type females to two white-eyed males. This is approximately the same ratio as that observed in the experimental results (45:55 wild type female to white eyed male ratio, with no other phenotypes present except for one mutant brown-eyed female).

- b) ~~Not~~
- | | | |
|-------|-----------|---------|
| | X^E | Y |
| X^E | $X^E X^E$ | $X^E Y$ |
| X^e | $X^E X^e$ | $X^e Y$ |
- 1:1:1:1 ratio, so the expected values are 25 for each phenotype (out of a total of 100).

$$\chi^2 = \sum \left(\frac{(o-e)^2}{e} \right) = \frac{4}{25} + \frac{36}{25} + \frac{9}{25} + \frac{1}{25} = \frac{50}{25} = 2$$

Since the value for the χ^2 equation is less than any of the values on the table for any # of degrees of freedom, it can be assumed that the deviations in

observed results from the expected results were due to chance, and not some

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