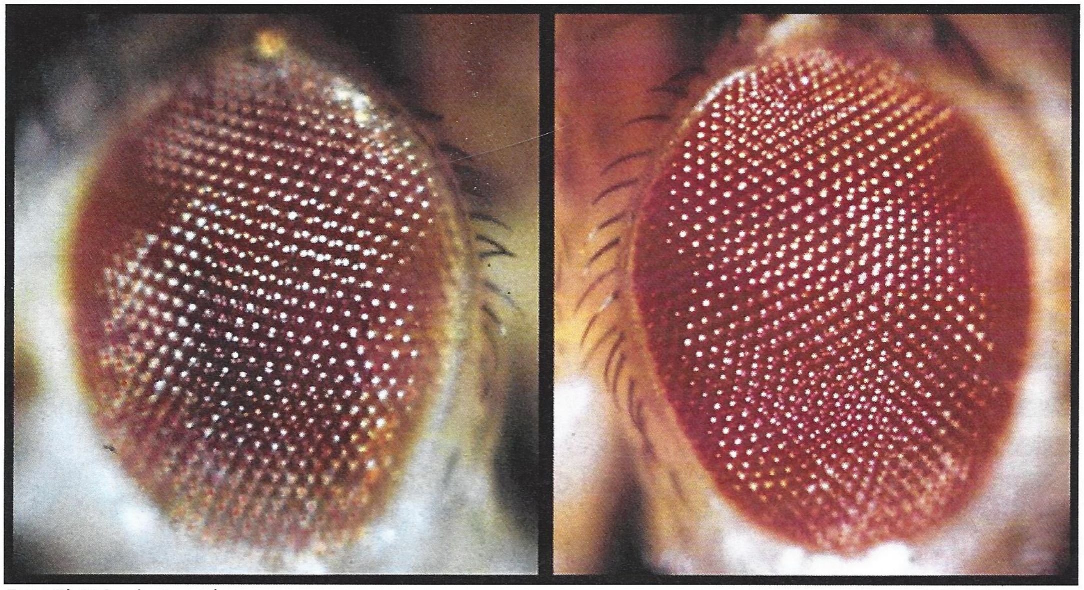
Easy Fly™ Dihybrid Cross Kit

TEACHER'S MANUAL AND STUDENT GUIDE



Easy Fly™ Sepia Female

Wild-Type Male

CAROLINA®

World-Class Support for Science & Math

Easy Fly™ Dihybrid Cross Kit

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Overview

Using this kit, students learn the basic principles of dihybrid autosomal inheritance and independent assortment using *Drosophila melanogaster*, an important model organism widely used for genetics and developmental biology research. Students observe how the apterous (wingless) mutation and the sepia eye color mutation are passed from one generation to the next. Then, they construct a hypothesis describing the mode of inheritance for each trait. This kit is designed for 32 students working cooperatively in eight groups of 4.

The Carolina *Easy Fly*™ system uses a modified Y* chromosome to selectively kill all male larvae during a 2-hour heat-shock treatment. The heat-shock treatment is performed prior to shipping, meaning that the *Easy Fly* sepia culture included in this kit will produce only virgin females. The ability to obtain a pure population of virgin females allows students to set up crosses with ease, and eliminates errors common in manual virgin collection. For general information on using the Carolina *Easy Fly* system, please refer to the General User's Guide included with this kit.

Content Standards

This kit is appropriate for high school students and addresses the following National Science Education Standards:

Grades 9–12

Unifying Concepts and Processes

- Systems, order, and organization
- Evidence, models, and organization
- Change, constancy, and measurement

Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Life Science

- Molecular basis of heredity
- Behavior of organisms

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Materials

Included in the kit:

- Order Form for prepaid delivery of the following *Drosophila* stocks (if ordered separately):
  - apterous
  - *Easy Fly* sepia
- FlyNap® Kit
- Formula 4-24® Instant *Drosophila* Medium
- 36 vials and plugs
- 72 vial labels
- 12 sorting brushes
- 100 sorting cards
- Carolina *Easy Fly™ Drosophila* Strains General User's Guide
- 9 copies of the Carolina™ *Drosophila* Manual
- Teacher's Manual and reproducible Student Guide

Needed, but not supplied:

- 8 stereomicroscopes (wide field microscopes)
- 2 sealable containers for morgues
- tap water and detergent (alternatively, 70% ethanol)

Storage Requirements

Store the cultures in a clean location not exposed to direct sunlight. Keep the culture vials at room temperature (20–25°C; 68–77°F). Lower temperatures slow the development of the flies. Higher temperatures promote male fly sterility, growth of bacteria and fungi, and mite infestation.

Using FlyNap®

Transfer the flies to an empty vial before anesthetizing them. To make the transfer, have an empty vial and foam plug ready. Tap the bottom of the culture vial (containing the medium and the flies) on the lab bench to knock the flies to the bottom. Quickly remove the plug of the culture vial and put the mouth of the empty vial over the mouth of the culture vial. Carefully invert the two-vial set and rap the bottom of the empty vial on the bench a few times to knock the flies down into the anesthetization vial. Take care not to knock the medium into the anesthetization vial. Quickly move the culture vial away and insert a plug into the anesthetization vial. Replug the culture vial.

After the flies have been transferred, tap them to the bottom of the anesthetization vial. Quickly insert a wand dipped in FlyNap into the vial, between the plug and the wall of the vial. Leave the wand in place until most of the flies have dropped to the bottom of the vial, and then remove it. When the flies are asleep (there may be some trembling of the legs and wings), put them on sorting cards for scoring. Do not leave the flies in the anesthetization vial for a long time. If FlyNap got on the plug when you inserted the wand, it will continue to anesthetize the flies after the wand has been removed and can kill them. If you plan to put all the flies into the morgue at the end of the lab, this will not matter. When returning anesthetized flies to a vial that contains medium, place the vial on its side so that the flies do not become stuck in the medium. Turn the vial upright after the flies recover. Flies can remain asleep for extended periods after being treated with FlyNap, so if the flies do not revive after a couple of hours, do not assume that they are dead. It may take 24 hours for some flies to recover.

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Background

See the Background section in the Student Guide for information about fruit flies as model organisms, and for details about sexing fruit flies.

Many students struggle to understand the concepts of dominant and recessive inheritance and the relationship between genotype and phenotype. Using Punnett squares can help students master these concepts. Present the following information to students as an introduction to (or a review of) the facility of using a grid to graphically predict the number and type of genotypes and phenotypes produced by a specific cross.

First, demonstrate a monohybrid cross of two homozygous flies, *WW* and *ww*. These homozygous adults constitute the Parental generation and produce the First Filial (F_1) generation.

Assign an uppercase letter to represent the dominant allele: *W*

Assign a lowercase letter to represent the recessive allele: *w*

Parental Cross Punnett Square

	W	*W*
w	*Ww*	*Ww*
w	*Ww*	*Ww*

F_1 Phenotypic Ratios

If dominant or recessive: 4/4 *Ww* (all carriers)

Next, cross two individuals of the F_1 generation. This cross produces the Second Filial (F_2) generation.

F_1 Cross Punnett Square

	W	*w*
W	*WW*	*Ww*
w	*Ww*	*ww*

F_2 Phenotypic Ratios

If dominant or recessive: 3/4 *W–*; 1/4 *ww*

Next, demonstrate the sex-linked mode of inheritance, as follows.

Sex-linked Parental: white-eyed female (*xx*) × wild-type male (*XY*)

Parental Cross Punnett Square

		Male	
		X	*Y*
Female	*x*	*Xx*	*xY*
	x	*Xx*	*xY*

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F_1 Phenotypic Ratios

If recessive, females are all wild type; males are all white-eyed.

Sex-linked F_1: wild-type females (*Xx*) × white-eyed males (*xY*)

F_1 Cross Punnett Square

		Male	
		x	*Y*
Female	*X*	*Xx*	*XY*
	x	*xx*	*xY*

F_2 Phenotypic Ratios

If recessive, for males, half are white-eyed and half are wild-type. For females, half are white-eyed and half are wild-type.

Dihybrid Crosses

After students have grasped basic monohybrid crosses, introduce a dihybrid Punnett square.

Assign uppercase letters to represent the dominant alleles: *R* and *W*

Assign lowercase letters to represent the recessive alleles: *r* and *w*

Dihybrid Parental Cross Punnett Square

	Rw	*Rw*
rW	*RrWw*	*RrWw*
rW	*RrWw*	*RrWw*

F_1 Phenotypic Ratios: 100% *R–W–*

F_1 Dihybrid Cross Punnett Square

	RW	*Rw*	*rW*	*rw*
RW	*RRWW*	*RRWw*	*RrWW*	*RrWw*
Rw	*RRWw*	*RRww*	*RrWw*	*Rrww*
rW	*RrWW*	*RrWw*	*rrWW*	*rrWw*
rw	*RrWw*	*Rrww*	*rrWw*	*rrww*

Alternatively, you may wish to introduce the branch method to help illustrate a dihybrid cross as an extension of the simple Punnett squares. Treat each locus separately. For example, if you cross two heterozygous individuals, *Rr* × *Rr*, expect a phenotypic ratio of 3 *R–*:1 *rr*, and the same for *Ww* × *Ww*. This is just like the F_2 ratios obtained in the monohybrid cross. The branch method simply multiplies these two monohybrid crosses to give the expected dihybrid ratio of 9:3:3:1.

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Branch Diagram

First Locus	Second Locus	Dihybrid Frequencies
$\frac{3}{4}$ *R–*	$\frac{3}{4}$ *W–*	$\frac{9}{16}$ *R–W–*
	$\frac{1}{4}$ *ww*	$\frac{3}{16}$ *R–ww*
$\frac{1}{4}$ *rr*	$\frac{3}{4}$ *W–*	$\frac{3}{16}$ *rrW–*
	$\frac{1}{4}$ *ww*	$\frac{1}{16}$ *rrww*

Chi-Square Statistics

The Chi-Square Goodness of Fit Test is a calculation that allows students to determine how likely it is that their observed data correlates with the expected data based on their hypothesis. Emphasize to students that the Chi-Square Test will not tell them *definitely* what mode of inheritance is at work, but will allow them to determine whether it is *plausible* that the observed data is consistent with a certain mode of inheritance. In other words, the Chi-Square Test will allow students to conclude whether their hypothesis should be rejected or not. The chi-square equation is:

$$\chi^2 = (\text{observed value} - \text{expected value})^2/(\text{expected value})$$

The Statistical Conclusion

In order for students to determine whether or not their chi-square value rejects or fails to reject their hypothesis, they must first determine the degrees of freedom (*df*) associated with their data. The number of degrees of freedom is a measure of how many categories are in the experiment. For genetic tests such as these, the number of degrees of freedom is one less than the number of phenotypic categories. For example, if there are two phenotypic categories (e.g., wild type and white), there is 1 degree of freedom (2 – 1 = 1). Based on the degrees of freedom, a probability of chance occurrence can be assessed by consulting a standard Chi-Square Distribution Table. Many professionals use a significance level of 5% ($p = 0.05$) to evaluate their experiments. If the probability of a chance occurrence is greater than 5%, then the hypothesis is not rejected. If the probability of a chance occurrence is less than 5%, then the hypothesis is rejected.

Preparation

1. Read the Teacher's Manual and the Student Guide thoroughly, and become familiar with the kit materials and activities before you begin the activities with your class.
2. **Order *Drosophila* Cultures (if ordered separately):** If this kit contains an order form for prepaid delivery of *Drosophila* stocks, submit the order at least 2 weeks before the requested delivery date. Consult the form to see how to submit your request via the Internet or by phone, fax, or mail.

   When the vials arrive, the *Easy Fly* sepia line will be ready to use. Refer to the Carolina *Easy Fly™ Drosophila* Strains General User's Guide included with this

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5. Have students set up F_1 vials by crossing virgin sepia females with apterous males. Each vial should have at least five male/female pairs. Excess flies should be disposed of in a morgue.
6. Discuss modes of inheritance with students, and have them write hypotheses for the modes of inheritance.
   a. When stating a hypothesis, one useful form is:

      The mode of inheritance for **(trait name)** in **(the organism of study)** is **(the type of inheritance tested)**.
   b. If a trait is autosomal recessive, all the F_1 flies will be wild type (red eyed or winged).
   c. If a trait is autosomal dominant, all the F_1 flies will display the mutant phenotype (sepia or apterous).
   d. If the sepia trait is sex-linked dominant, all the F_1 flies will display the mutant (sepia) phenotype. This is because homozygous sepia females are used for this cross, and females pass their X chromosome to F_1 males and females.
   e. If the apterous trait is sex-linked dominant, all the F_1 females will display the mutant (apterous) phenotype. This is because apterous males are used for this cross, and males pass their X chromosome only to F_1 females.
   f. If the sepia trait is sex-linked recessive, all the F_1 males will display the mutant (sepia) phenotype and all the females will display the wild-type (red eye) phenotype. This is because sepia females are used for this cross.
   g. If the apterous trait is sex-linked recessive, all the F_1 flies will display the wild-type (winged) phenotype. This is because winged, wild-type females are used for this cross. Similarly, if the apterous trait is autosomal recessive, all the flies will display the wild-type (winged) phenotype. Students will not be able to determine whether apterous is a sex-linked or autosomal recessive trait until the F_2 generation is scored.

Week 2: Clearing the F_1 Vials

15-minute activity. Conduct 5–7 days after setting up the parental cross.

1. Have students anesthetize the Parental generation flies in the F_1 vial using the provided FlyNap kit and instructions.
2. Remove all adult flies from the vials in order to prevent generational crossover, and dispose of them in a morgue.

Week 3: Scoring the F_1 Flies and Setting Up the F_1 Cross

45-minute activity. Conduct 5–7 days after clearing the F_1 vials.

1. Have students anesthetize the F_1 flies using the provided FlyNap kit and instructions.
2. Have students score the F_1 flies. Data for F_1 scoring should include the date and number of males and females for each phenotypic category.

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3. Prepare, or have students prepare, one F_2 vial for each group with media and food, as was done in Week 1.
4. Have students transfer five randomly selected F_1 males and five randomly selected F_1 females to the F_2 vials to establish the F_1 cross. This cross will produce the F_2 generation.
5. Assist students as they compare and contrast their two hypotheses for the modes of inheritance for apterous and sepia. Help students make predictions based on their hypothesis for the genotypes and phenotypes of the F_2 generation (see Expected Results).

Week 4: Clearing the F_2 Vials

15-minute activity. Conduct 5–7 days after setting up the F_1 cross.

1. Have students anesthetize the F_1 flies using the provided FlyNap kit and instructions.
2. To prevent generational crossover, remove all adult flies from the vials and dispose of them in a morgue.

Weeks 5 and 6: Scoring the F_2 Flies

15-minute activity. Conduct 5–7 days after clearing the F_2 vials. Flies should be scored every other day for 7 days.

Note: As an alternative to scoring the F_2 flies every other day for 7 days, students may score the flies once, 10–14 days after clearing the F_2 vials in Week 4. This approach consolidates scoring into one laboratory session, but there will be many more flies to score at one time, increasing the chance of scoring errors and escaping flies. This approach should yield phenotypic proportions accurate enough to determine the modes of inheritance.

1. Have students anesthetize the F_2 flies using the provided FlyNap kit and instructions.
2. Have students score the F_2 flies. Data for F_2 scoring should include the date and number of males and females for each phenotypic category.
3. Have students dispose of the flies in a morgue.
4. **Optional:** Have students make a statistical conclusion using chi-square analysis. The Appendix provides additional chi-square background information and a worksheet.

Expected Results

From observation of the F_1 phenotypes, students should note that all F_1 males and females display the wild-type phenotype for both characteristics (red eyes, winged). This pattern indicates that sepia is inherited as autosomal recessive. (Because mutant sepia females were used in the cross, if this mutation were sex-linked recessive, the F_1 males would show the mutant phenotype). For the apterous mutation, the F_1 observations are consistent with either autosomal or sex-linked recessive (see diagram on next page). Ask the students to determine how

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these options can be distinguished by examining the F_2 generation. To accomplish this, students should model the inheritance of this trait for both options. The two models predict different outcomes in the F_2 generation. The two outcomes are displayed in the hypothesis flow charts.

Based on their observations of the F_1 generation, students should generate one of two hypotheses for the mode of apterous inheritance. Hypothesis A states that the trait is autosomal recessive. Hypothesis B states that the trait is sex-linked recessive.

Hypothesis A: The mode of inheritance for apterous in *Drosophila* is autosomal recessive. (Correct) **Key:** wild-type (*wt*), apterous (*ap*)	**Hypothesis B:** The mode of inheritance for apterous in *Drosophila* is sex-linked recessive. (Incorrect) **Key:** wild-type (*wt*), apterous (*ap*)
In this case, the parental cross is *wt/wt* females × *ap/ap* males. ↓ The F_1 is comprised of *wt/ap* males and females. ↓ The F_2 will exhibit a ratio of 3 wild type:1 apterous both for males and females.	In this case, the parental cross is wild-type females × apterous males. ($X^{wt} \times X^{ap}$) ↓ F_1 X^{wt}/X^{ap} females X^{wt}/Y males ↓ The F_2 females all are wild type. The F_2 males exhibit a ratio of 1 wild type:1 apterous.

In the F_2 generation, students will observe that the sepia and apterous characteristics both reappear in independent 3:1 ratios that are not skewed by sex, confirming the hypothesis of autosomal recessive inheritance for both traits. This result also demonstrates that the two traits assort independently.

Sample Chi-Square Analysis

For this example, the hypothesis has an expected phenotypic ratio of 9:3:3:1.

The null hypothesis is that the difference between expected value and observed value is not significant.

Step 1: On the basis of your hypothesis, determine the ratio of phenotypes you *expected* in the F_2 generation. In this example, 192 flies are observed.

Phenotype	Expected Ratio	Expected Number of Flies ("e")
1. red eye (wild-type)/winged (wild-type)	9/16	108
2. sepia eye/winged (wild-type)	3/16	36
3. red eye (wild-type)/apterous	3/16	36
4. sepia eye/apterous	1/16	12

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Step 2: Record the ratio of phenotypes you *observed* in the F_2 generation.

Phenotype	Observed Ratio	Observed Number of Flies ("*o*")
1. red eye (wild-type)/winged (wild-type)	112/192	112
2. sepia eye/winged (wild-type)	30/192	30
3. red eye (wild-type)/apterous	41/192	41
4. sepia eye/apterous	9/192	9

Step 3: Complete the following table using data from the entire class. For "*e*" and "*o*" values, use the actual numbers of flies rather than ratios or percentages.

Phenotype	1. red eye/ winged	2. sepia eye/ winged	3. red eye/ apterous	4. sepia eye/ apterous
Expected Value "*e*"	108	36	36	12
Observed Value "*o*"	112	30	41	9
Deviation (d) = $o - e$	4	–6	5	–3
Deviation Squared (d^2)	16	36	25	9
$d^2 \div e$	**0.148**	**1**	**0.694**	**0.75**

Add all the $d^2 \div e$ values together to calculate the χ^2 value.

χ^2 = **2.592**

Step 4: Calculate the degrees of freedom by subtracting 1 from the number of possible phenotypes.

phenotypes possible = **4** – 1 = **3**

degrees of freedom = **3**

Step 5: Decide whether to accept or reject your hypothesis. Using the table below, determine the probability that the deviation is due to chance.

Chi-Square Distribution Table

Degrees of Freedom	Probability of a Chance Occurrence							
	90%	70%	50%	30%	20%	10%	5%	1%
1	0.016	0.148	0.455	1.074	1.642	2.706	3.841	6.635
2	0.211	0.713	1.386	2.408	3.219	4.605	5.991	9.210
3	0.584	1.424	**2.366**	**3.665**	4.642	6.251	7.815	11.341
4	1.064	2.195	3.357	4.878	5.989	7.779	9.488	13.277

χ^2 = **2.592**

The probability that the difference between the expected value and observed values was caused by change is between 30% and 50%. Because this probability is above the standard 5%, we do not reject our hypothesis.