Drosophila Experiments for High School Biology

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The common fruit fly, Drosophila, is an excellent organism to use in demonstrating inheritance. During the course of one semester, several generations of these flies can be obtained. By following proper breeding procedures, it is possible to verify many basic rules of heredity. In addition, students may learn a number of other biological facts and techniques by working with living cultures of these flies.

Drosophila are really very easy to raise. They flourish at ordinary room temperature and complete their life-cycle in about 12 days. They may be anesthetized for handling and sorting. Many types of hereditary variations can be classified with the naked eye. Table I lists easily identified Drosophila types.

New and interesting experiences await the teacher and student as they work with each new characteristic. Breeding experiments may be conducted in which the principles of heredity are disclosed. Histological studies will reveal the ontogeny of normal and mutant types. Various components of the environment (such as temperature) may be changed to show basic principles of physiology and ecology.

Handling Flies

Flies can be anesthetized for examination and transfer. An ether chamber for this purpose may be made using the same type of bottle in which the stocks are kept. Stopper the bottle with a tight fitting cork to which has been attached a bit of cotton or other absorbent material. A few drops of ether on the cotton is sufficient to fill the chamber with fumes.

To remove the flies from a rearing bottle, tap bottle gently on table top to dislodge flies from the cap end of the bottle. Quickly remove cap and place ether chamber over open bottle. Invert bottle and chamber together and shake flies into chamber by tapping on palm of hand. Quickly close the chamber with the cork and ether soaked cotton. Practice this several times with an empty bottle before attempting to do it with live flies.

TABLE I

Hereditary traits in Drosophila which may be identified with the naked eye. (Wild-type, +, is always dominant except with Bar. All other genes are recessive.)

	Symbol	Name	Description
Wing types:	+	Wild-type	Normal wing
	vg	vestigial	wing reduced to stub
	m	miniature	wing half-size (sex-linked)
	c	curved	wing curved down
	cu	curled	wing curved up
	ho	held-out	wings spread
	rsd	raised	wings held erect
	tx	taxi	wings divergent
Body colors:	+	wild-type	tan-gray color with black accents
	e	ebony	black body
	y	yellow	light yellow body (sex linked)
Eye types:	+	wild-type	red eye color
	w	white	colorless eye (sex-linked)
	se	sepia	dark brown eye, almost black
	cl	clot	dark brown eye
	B	Bar	eye reduced to narrow stripe (sex-linked)
	ey	eyeless	eye reduced to small dot

Preparation of Drosophila Media

The following recipe will make enough media for about 30 rearing bottles (½ pint cream bottles).

Water	1000 cc.
Agar	18 gm.
Corn Meal	60 gm.
Dry Yeast	10 gm.
Drosophila mix*	50 gm.
Propionic Acid**	5 cc.
Methyl Parasept** (12.5% soln. in alcohol)	12 cc.

Mix agar, water, and corn meal and cook 10-15 minutes, stirring frequently. Add extra water if too thick. Add Drosophila mix and cook at a slow boil for 2 minutes longer. Cool 5 minutes and stir in propionic acid and methyl parasept. Pour to a depth of about 1 inch in clean rearing bottles being careful not to let food run down sides of bottle. Cap the bottles immediately to prevent stray flies from entering and depositing eggs. Bottles may be stored at room temperature but should be protected from heat or extremely dry conditions.

*Drosophila mix may be made up in advance and kept on shelf, as follows:

Sugar (Sucrose)	300 gm.
$NaNO_3$	30 gm.
K_2HPO_4	10 gm.
$MgSO_4$	5 gm.
KCl	5 gm.
$FeSO_4$	0.1 gm.

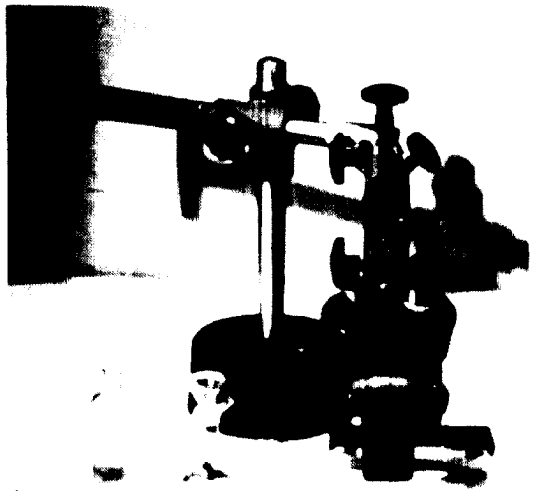
**Mold and bacteria inhibitors. Methyl Parasept may be obtained from Heyden Chemical Corp., 393 Seventh Ave., New York 1, N. Y.

The flies will succumb in about 10 seconds after they encounter the ether fumes. When all flies are motionless (after about 15 seconds), remove the cork and pour flies on a clean 3 x 5 white card for sorting and examination. If flies are allowed to remain in the etherizer too long, their wings bend upward, their bodies curl and they die.

Etherized flies will recover in about 5 minutes. Sorting and examining, therefore, must be done rapidly and with small lots of flies. Flies are usually rendered sterile by etherizing a second time.

Sorting can be done with a pocket knife or sharp dissecting needle. String the flies along the card in a thin line and sort from right to left (if you are right handed) by sweeping one type toward the top of the card and another toward the bottom.

Care should be taken not to overheat the flies with a strong light while examining them. Fluorescent lights should be used if the light source is to be brought close to the flies.



Typical microscope set-up for sorting and counting flies. A less elaborate microscope can be used. With many traits, identification and sorting can be done without the aid of a microscope.

Rearing

Before introducing flies to a fresh food bottle, seed the surface of the food with a few grains of live yeast (small, foil-wrapped packages may be obtained at your grocery store) or a few drops of a suspension of live yeast. The food in the bottle is primarily for growth of yeast, upon which the flies feed. Next, insert a piece of tissue or paper toweling about 2 inches square into the food to provide a dry surface upon which the flies may be dropped. Flies will become stuck and die if dropped on the surface of the moist food.

Rearing bottles must be kept at a temperature no higher than 80 degrees F. and, preferably, above 70 degrees F. They should not be exposed to direct sunlight and may be kept entirely in the dark.

About 5 days after introducing flies to a fresh bottle, small larva may be seen crawling in and over the surface of the food. In about 8 days, pupa will be evident along the edges of the piece of tissue. A new generation of adults will begin to emerge from the pupa cases in about 12 days.

Identification

The easiest way of distinguishing male and female is by reference to the posterior end of the abdomen. The male appears to have a black tipped, blunt posterior end, while the

Wild-type flies. Male above, female below.

Held-out, right, and normal wing types.

female is lighter colored and has a pointed posterior. Looking on the ventral surface, the male genetalia serve as a distinguishing feature.

Obtaining Virgin Females for Studies of Heredity

Select a rearing bottle in which new adults are emerging in considerable numbers (i.e. a culture about 14 days old) and remove *all* adult flies from the bottle and discard or transfer to a new bottle. Make a note of the time when this is done. Return to the bottle in 10-12 hours and remove all adults that have emerged in the interim. The females thus obtained will be virgins and may be used in making crosses. Females older than 12 hours, if they have been with males, should be considered mated. Once mated, a female may not be successfully mated to another male. Pale colored flies with incompletely expanded wings are those which have just recently emerged from the pupa case. These should be avoided in selecting flies for a cross since ether will sterilize flies of this age.

Making a Cross

Select virgin mutant (vg) females and place 2 in each of 3 food bottles. Place with them two wild-type (+) males. Mark the date and the type of mating on the cap. They will mate soon after recovering from the ether and the female will begin to lay eggs in about 36 hours. Thereafter, she will lay about 50 eggs a day. Matings are made in triplicate in order to assure success.

After seven days, remove the parent flies from the mating bottle and discard them. By this time, larva should be evident in considerable numbers.

The Hybrid (F_1's)

About 12 days after the date of the original mating, F_1 flies should begin to emerge. The first flies will be almost all females because they have a slightly shorter developmental time than males. These F_1's should be all wild-type (+) flies. If any mutant (vg) flies emerge at this time it is an indication that the parent female was not virgin.

Place about 6 pairs of F_1 flies in each of 3 fresh food bottles (these need not be virgin flies). Mark these with the date, the type of original mating, and the generation. Each of these food bottles will produce about 250 flies in the next generation.

Virgin F_1 females may be obtained and back crossed to mutant males to provide an interesting back cross generation for counting.

The F_2 Generation

The 6 pairs of F_1 flies should be removed from each of the bottles in about 7 days. In the usual length of time, the F_2 generation will begin to emerge. The sex and type of each of the F_2 flies should be determined and recorded. Flies will continue emerging for

Close-up side view of anesthetized female. Notice the curled wings, short blunt bristles, bar eye and white eye color. All these traits are inherited.

Close-up of Drosophila head showing bristle placement. Presence of two large bristles on mid-posterior of head depends on a single hereditary factor.

about 8 days. Countings should be made every other day.

Questions

1. Why are the F_1's all wild-type?
2. Was there any difference in the ratio of mutant types to wild-types in the first count and the third count of the F_2? What about the ratio of males to females in different counts?
3. What is the grand total ratio of mutant types to wild-types?
4. Is a backcross ratio different from an F_2 ratio? Why?
5. Is the ratio of mutant females to wild-type females the same as the ratio of mutant males to wild-type males?
6. Would a mating of wild-type females and mutant males give any different results? (Why not try it?)

Obtaining Pure Cultures

Pure cultures of various Drosophila mutants may be obtained by mail from several different biological supply houses. They cost about $2.75 each. It is also possible to obtain ready-made crosses in large bottles for a slightly higher price.

Once obtained, the pure stocks may be carried on by transferring a few flies to a new media bottle about every three weeks. Stocks may be lost during the summer if provision is not made to keep them cool.

Interesting Projects

1. *Sex-linkage.* Cross Bar female with wild-type male. In F_1, all males will be Bar and all females, wide-Bar (indicating heterozygous B). If wide-Bar F_1 females are crossed to wild-type males, half the male progeny will be Bar and half wild-type; half the females will be wide-Bar and half wild-type.

If the original cross is made with wild-type females and Bar males, the F_1 males are (like their mothers) all wild-type and the F_1 females are all wide-Bar.

This experiment shows the pattern of sex-linked inheritance a little more clearly than one in which a recessive is used. Since the Bar gene is dominant, its presence is never "hidden" in the heterozygous condition.

2. *Autosomal dihybrid inheritance.* Cross a vg female with an e male (or vice versa). The F_1 will be all wild-type and should be mated *inter se* to give and F_2. The F_2 should yield a ratio of 9 wild-types, 3 vg, 3 e, and 1 vg e. It is interesting to take virgin F_1 females and "testcross" mate them to vg e males to show a typical testcross ratio of 1 wild-type, 1 vg, 1 e, and 1 vg e.

3. *Linkage and crossing-over.* Cross ho cl female with a wild-type male and testcross both F_1 females and F_1 males to the ho cl stock. The testcross of F_1 female to ho cl male should give a ratio of about 9 wild-type, 1 ho, 1 cl, 9 ho cl (very different from the ex-

Curled wings, also with bar eye and white eye color.

pectel 1:1:1:1 if these genes were not linked). When the F_1 male is used to testcross to a virgin ho el female, the progeny are half ho el and half wild-type, indicating a unique feature of Drosophila inheritance, namely that crossing-over does not take place at all in the male.

4. *Life cycle.* Raise Drosophila at several different temperatures, perhaps near a cold window, near the ceiling, on the floor, and on a table. Have a thermometer near each site to record the temperature. Carefully observe the length of each stage in the life cycle (egg, larva, pupa, adult), and relate this to the temperature. In general, one finds that the life cycle lengthens as the temperature goes down.

Reference

"Drosophila Guide." M. Demerec and B. P. Kaufman. Published by Carnegie Institution of Washington, 1530 P Street Northwest, Washington 5, D. C. and sold for 25 cents a copy.

OHIO SCIENCE FAIR GROUP SOLVES 'HOPPER PROBLEM

ARCHBOLD, Ohio—One way to tackle the grasshopper problem is to serve them French-fried to guests.

This was the tasty treat in store for visitors to the 5th Annual Quadri-County Science Fair held at the high school here (April 8, 9). Described as both "edible and palatable," the hot 'hoppers were whipped up by the Epicures Club as a fair treat.

Last year, the same group of gourmets passed out tasty little tidbits of rattlesnake meat to those who came to view the science exhibits.

Science Service

Special Techniques for Growing Ferns

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The sensitive fern, *Onoclea sensibilis*, has erect fertile stalks that are easy to locate in moist soil during the winter. In this particular fern one may speed the release of the spores by placing the sporophyll heads for one second (in and right out) of boiling water. Then the stalks are dried by laying them on a flat sheet of paper in the laboratory or, if one prefers, into a low temperature (37°C) oven. Within one to two days many of the spores will be released (Fig. 1). One may carefully hold the paper containing the spores at an angle near enough to the vertical to let most of the heavy spore cases fall, while most of the spores will be retained. The spores are placed on the surface of mineral solution (1) in a petri dish; here they will grow in ten days into prothallia which may be used for class

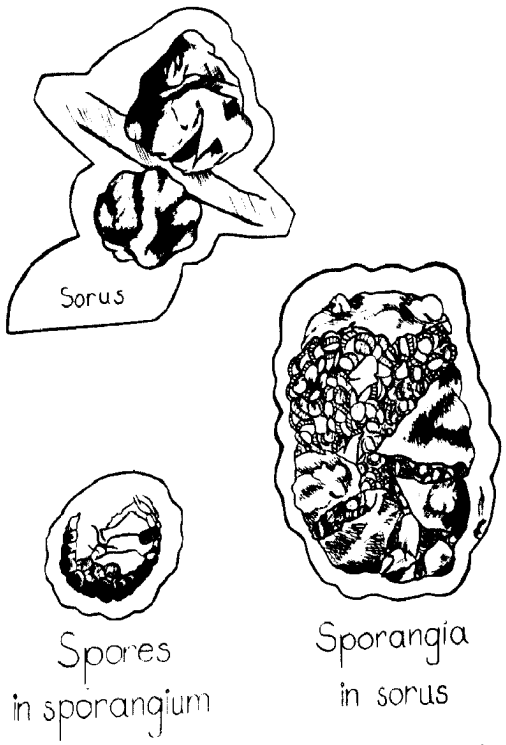

FIGURE 1—The reproductive structures of sensitive fern are sketched from live material under 20X magnification. Drawings by George Mustric, eleventh-art student.