APPENDIX

Methods of Breeding Drosophila

Drosophila ampelophila has shown itself to be so generally useful for class work in genetics and is being so widely employed for this purpose that it may not be out of place here to give a few directions concerning apparatus, methods and material.

Culture Bottles.—Large-mouthed bottles of about 500 c.c. capacity should be used. Pint milk bottles can be purchased at reasonable rates from wholesale dealers, and serve admirably as culture bottles. Stoppers of raw cotton are used, which should be tight but not packed in the mouth of the bottle.

Temperature.—The optimum is about 25° C. Extreme summer heat kills the flies in culture bottles unless special precautions are taken. Cold retards the development of the larvæ indefinitely, but the flies themselves can withstand almost a freezing temperature. Ordinary room temperature suffices, as a rule, but a controlled temperature of about 25° C. is better.

Food.—Ripe fermented banana is the best food. If raw bananas with intact skin are peeled and put into old juice contamination is not likely to occur, but for ordinary purposes the bananas should be peeled, broken up into pieces, covered with water, and slowly brought to nearly the boiling point, or else steamed. The pieces are then put into the old juice. This juice is made in the first instance by adding a little yeast to the water that covers the bananas (after they have cooled). The juice can then be used over and over again if it is occasionally greatly diluted with water to keep it from becoming too acid. The food is at its best from one to two days after being in the juice, although it may be used for a week or more. Keep food in glass-stoppered large-mouthed jars. Scrupulously avoid leaving the margin of the jar wet after removing food.

FEEDING.—When a culture is to be made up the most approved way is to put about a teaspoonful of food on the bottom of the bottle and over it a folded piece of absorbent paper. The flies, while still under ether, may be dropped into a cornucopia of paper which is placed in the bottle. Often, however, the flies are brushed into a dry bottle and the cotton plug put in. An hour or more later, when the flies have recovered, the food, wrapped up in paper, is added. Toweling paper is cheap and serves excellently the purpose of wrapping the food, etc.

By putting in at first more food than recommended above it is possible to carry a culture to the end without further feeding. But more flies and greater accuracy result if a small amount of food is first added, then as much more at the end of six or seven days. When the parent flies are taken out at about the tenth day, food may be added for a third time. New food should not be allowed to cover the old food and thereby drown the pupæ.

PRECAUTIONS.—In most cases a single female with one, or in some cases more males should be put into a bottle, except for stock breeding, when more flies should usually be used. Under favorable conditions 200 to 300 flies should be obtained within the first ten days after hatching has commenced. The parents should be removed about ten days after the beginning to avoid overlapping of generations. If desirable they may then be transferred to a second, or even to a third culture bottle. In summer the parents should not remain over eight days in the old bottle; in winter they may be left more than ten days with safety. To obtain virgin females for mating, the bottles should be thoroughly emptied—it may be necessary to remove the old paper, etc., in order to make certain that all of the old flies are removed—and females obtained not later than six hours after the bottle was emptied. Females obtained twelve hours after the bottle was emptied are not certainly virgin. If the old males have not been removed females so young as to have their wings not yet unfolded may in rare cases have already undergone copulation.

EXAMINATION.—The flies go toward the light. Therefore, if the bottle is held with its mouth away from the light, the flies are not likely to escape. The plug may then be removed and another smaller bottle, with a mouth that fits the larger one, placed mouth to mouth against the larger bottle. The combination is then turned around, and the flies fly into the smaller bottle—or the smaller bottle may be held firmly underneath the other and the flies shaken into it by jarring. The small bottle can then be plugged, and a bit of cotton with four or five drops of ether put into it. In a minute or two the flies are under the influence of the ether and may be emptied out on to a piece of white paper, or a white glass plate. Some workers prefer to have the small bottle already saturated with ether before the flies are shaken into it; in this case they become etherized almost immediately. They can then be examined with a hand lens or with a binocular microscope. Some of the characters require for study the latter or an ordinary microscope.

With a camel's hair brush the flies are pushed out into a row and then sorted out, from right to left, into an upper and a lower row, each of which may again be subdivided. If overetherized, the wings stand out above and at right angles to the body. If insufficiently etherized, so that they recover before they can be examined, they may be etherized again.

The pure stock is kept in pint bottles and new cultures made up each week. Descriptions of the mutants as far as published will be found in the following journals:

Bar.—"A New Sex-linked Character in Drosophila." *Biol. Bull.*, XXVI. 1914.

Beaded.—"The Analysis of a Case of Continuous Variation, Etc." *Amer. Nat.*, XLIII. 1914.

Bent.—"A Gene for the Fourth Chromosome of Drosophila." *Jour. Exper. Zoöl.*, XVII. 1914.

BLACK.—"Heredity of Body Color in Drosophila." *Jour. Exper. Zoöl.*, XIII. 1912.

CHERRY.—"A New Eye Color Mutation in Drosophila, Etc." *Biol. Bull.*, XXV. 1913.

EBONY.—"A Third Group of Linked Genes in Drosophila." *Science*, XXXVII. 1913.

EOSIN.—"Dilution Effects and Bicolorism, Etc." *Jour. Exper. Zoöl.*, XV. 1913.

EYELESS.—"Another Gene in the Fourth Chromosome of Drosophila." *Amer. Nat.*, XLIX. 1915.

LETHAL.—"Two Sex-linked Lethal Factors in Drosophila, Etc." *Jour. Exper. Zoöl.*, XVII. 1914.

MINIATURE.—"A Modification of the Sex Ratio, Etc." *Zeits. f. ind. Abst.- u. Vererb.-Lehre.*, VII. 1912.

PINK.—"Dilution Effects and Bicolorism, Etc." *Jour. Exper. Zoöl.*, XV. 1913.

RUDIMENTARY.—"A Modification of the Sex Ratio, Etc." *Zeits. f. ind. Abst.- u. Vererb.-Lehre.*, VII. 1912.

SPOT.—"Another Case of Multiple Allelomorphs in Drosophila." *Biol. Bull.*, XXVI. 1914.

VERMILION.—"Dilution Effects and Bicolorism, Etc." *Jour. Exper. Zoöl.*, XV, 1913.

VESTIGIAL.—"No Crossing Over in the Male of Drosophila, etc." *Biol. Bull.*, XXVI. 1914.

WHITE.—"Sex Limited Inheritance in Drosophila." *Science*, XXXII. 1910.

YELLOW.—"Heredity of Body Color in Drosophila." *Jour. Exper. Zoöl.*, XIII. 1912.

FORMULÆ

Baur's plan of using non-significant letters has no doubt certain advantages, but in practice significant letters are too useful to be given up. We have followed a plan which avoids the objections of the presence and absence scheme, and has the advantage of significant letters. In this plan a small letter is used for the mutant factor if recessive, and a large letter if dominant. With a little practice we have found, from our own experience, there is no real difficulty in making the transition from the presence and absence notation to this one. For example:

	Simplex	Duplex
Pink eye	pVW →	$\frac{pVW}{pVW}$
Vermilion eye	PvW →	$\frac{PvW}{PvW}$
White eye	PVw →	$\frac{PVw}{PVw}$
Red eye	PVW →	$\frac{PVW}{PVW}$

A further simplification would consist in using the letters for the mutant factors alone, as Castle has done, and omitting the normal factors. But in writing out formulæ for heterozygous forms, it is often convenient to represent both members of a pair of allelomorphs. In matters relating to linkage it is essential to indicate, in some way, both allelomorphs.

If in any formula it is desirable to distinguish between dominant and recessive mutant factors, it may be convenient to prime both allelomorphs of a pair in which the factor is named from the dominant character.

In addition to the more important objections to the presence and absence representation that have been dealt with in the text, there are certain technical drawbacks to the presence and absence scheme of nomenclature that should not pass unnoticed.

When to a familiar or to an established system it becomes necessary to add new recessive types difficulties arise. This may be illustrated in the case of combs of fowls, the main facts concerning whose inheritance have been discussed on page 216. On the presence and absence scheme a factor gets its name from the effect that that factor produces in the absence of other factors affecting the character. The factor for pea, for instance, got its name from the effect produced when a factor for rose was supposed to be absent; and the formula for single comb, rpS, means that a factor S, for single, produced its particular effect when the two other factors were absent. When a new condition, combless, was met with and added to the series it was represented as due to the loss of the factor (S) for single. The formula for combless became rpsB (B standing for the vestige of a comb, called Breda, that remained). What now is the factor for rose comb? Originally this factor got its name from its effect in the absence of pea but in the presence of S (RpS); now the factor for rose, R, should be re-named from its effect *in the absence of both* factors P and S. The series must then be re-constructed on a new basis and the same process must be gone through with whenever a new factor is brought into relation with an established system.

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