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Sex-Linkage in Drosophila Melanogaster with Mention of Mutation Factors

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SEX-LINKAGE IN DROSOPHILA MELANOGASTER

WITH MENTION OF MUTATION FACTORS.

Submitted in partial fulfillment of requirements for honors

in Biology.

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O U T L I N E

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A - Early work on heredity and sex-linkage

B - Experimental Materials and Work

1 - Preparation of materials

11 - Satisfactory culture medium

111 - Handling of flies

1V - Matings

C - Results

D - Mutations

E - Summary

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All work on heredity must be traced back to Gregor Mendel. His careful, painstaking work in trying to discover the law of inheritance in hybrid varieties has given us the starting point for all subsequent work in the field of heredity, i.e. Mendel's Law: independent unit characters, dominance, purity of gametes (segregation). With this law as a basis or starting point, T. H. Morgan, Professor of experimental zoology at Columbia University, made the first attempt - about 1900 - to try to find out the mechanism of sex-linkage. Morgan used as his subject, the same insect which I have used - Drosophila melanogaster, or common fruit fly - and he also made use of the red and white eye colors. Morgan at first believed white eye to be a secondary sexual characteristic in males, but when he realized it could be introduced into females by selective breeding, he recognized it as being a recessive characteristic and making its appearance in female only when occurring in a double linkage as worked out by Mendel. From his results, Morgan determined that the genes of sex-linked characteristics are carried in the X-chromosome, and that the Y or male chromosome does not carry genetic characters; this fact accounts for the appearance of recessive characteristics in males more so than in females, for in a male there is only one X-chromosome, while in a female there

are two. Through his work, Morgan also found that females tend to inherit characteristics of the father, while males tend to inherit characteristics of the mother, because sex-linked characteristics are carried by the X-chromosome alone.

There are many sex-linked characters in Drosophila melanogaster including eye color (several types), bar eye, wing length, body color, wing shape, etc. For my work, I chose the dominant red and recessive white eye colors, and essentially I used crosses similar to those of Morgan.

The materials necessary for breeding and examining cultures of Drosophila melanogaster are relatively few, and are quite inexpensive. About 20-25 wide mouthed bottles of 250 cubic centimeter capacity and stoppers for these bottles are needed; in addition to these, one needs a camel's hair brush, a small light capable of fairly bright illumination, a small quantity of ether, an etherizing bottle, a Petri-dish etherizer, a hand lens (the usual type upright microscope is not suitable for use here because of the small objective field), and material for making a suitable culture medium. A good stopper for the above mentioned bottles can be made by wrapping a small amount of cotton in a piece of cheesecloth or gauze; this gives a plug for the bottle opening which will allow air to enter and at the same time will keep impurities out.

Before using, all materials which will be in contact with the flies should be sterilized. This may be accomplished by washing the bottles in a dilute phenol solution, and then subjecting both bottles and stoppers to 30 lb. per square inch pressure of steam for thirty minutes in an autoclave. As

soon as possible after materials are removed from the sterilizer, the stoppers should be put in the bottles to prevent entrance of impurities. (See Footnote)

The next step in getting materials ready for use - is the making of a suitable food medium for the larvae. There are many good media in which to breed Drosophila melanogaster, including the agar, molasses (free from H_2SO_4), and corn meal mixture in which the flies I used were shipped to me, but I have found that a mixture of 5% agar, bananas and yeast is most suitable - - by using this mixture, flies are not killed by becoming stuck in the medium, as often occurs with the molasses mixture. For my culture medium, I mixed half a banana, crushed into a pulp, 300 cubic centimeters of water and 15 grams of agar; this mixture was heated over a period of 30 minutes (slowly to boiling) with frequent stirring to prevent burning, and poured while hot into bottles to a height of 3/4 inches. After this mixture had cooled, a few grains of dried yeast - to

Footnote: Mold is one of the most destructive influences for which the breeder must be on the lookout. Mold spores are floating in the air at all times, and if care is not taken in sterilization of materials and preparation of culture medium, the mold will develop in the breeding bottles. Mold which was present in the vials in which the original flies were received caused me to lose all of my original cultures, not to mention a great deal of time. Two kinds of mold were evident - a black type which developed in circular spots, and a fine green type which spread over the whole culture medium; this latter type grew on the flies at the junction of thorax and abdomen and spread from there until it killed them.

assist in breaking down complex carbohydrates into more simple form for the larvae to eat - were added to each bottle, and also a strip of paper toweling on which the pupae could affix themselves.

One of the 250 cubic centimeter bottles may be used as an etherizing bottle; this should be equipped with a tightly fitting cork stopper to the under side of which a ball of cotton has been nailed. When this bottle is ready for use, the cotton ball is saturated with ether and the cork stopper is placed in the bottle. Care must be taken that the flies are not killed by an overdose of ether; $1\frac{1}{2}$ - $2\frac{1}{2}$ minutes of exposure to strong ether fumes should be enough to render the flies insensible. After the flies have been anaesthetized, they may be dropped out on a piece of white paper for examination. The camel's hair brush will be found quite useful in turning and examining the flies. If during examination, the effect of the ether wears off, the petri-dish etherizer should be brought into use. This instrument may be made by fixing cotton to the center of a petri-dish by means of gummed paper, and saturating the cotton with ether. If flies show signs of activity while being examined, the petri-dish may be placed over them, and the ether fumes will soon overcome them again. This avoids the necessity of returning the flies to the etherizing bottle when they recover.

Drosophila melanogaster are positively phototropic; this characteristic may be made use of in facilitating transfer of flies from a culture bottle to an etherizing bottle. After the removal of the cotton plug from the culture bottle, the mouths

of the two bottles are quickly brought together with the base of the etherizing bottle toward a source of strong light. It will be found that the flies will immediately move toward the light, and thus pass from the culture bottle into the etherizing bottle where they may be anaesthetized for examination. Newly hatched flies will be quite sluggish and may be slow in moving toward the light because they have not secured full use of their wings. About 15-20 hours after hatching is the best time to effect transfer and examination of flies as by this time full use of wings is acquired and sexual characteristics have become quite definite.

On Page 13, there are drawings showing sexual differences in Drosophila melanogaster, but I think it suitable at this point to describe some of the characteristics which help in differentiating the sexes.

* "Males, in general, are smaller than the females. Females emerge from the pupae six hours before the males, thus the first flies hatching in a culture tend to be females."

(1) The easiest distinguishing characteristic is the shape and coloration of the abdomen.

a. MALE - tip of abdomen is rounded, two last segments solid black in dorsal view. (The mutant yellow has a dark brownish color).

b. FEMALE - tip of abdomen pointed, all segments intensely colored on posterior rim of tergites only.

* From Paper sent by Ward's Natural Science Establishment, Inc., Rochester, N.Y.

(2) In newly emerged individuals, the shape and color are not so easily distinguishable, and for this reason, these characteristics are not as useful in differentiating between the sexes. A ventral view shows the following differences which can also be used in older flies.

a. MALE the external genitalia are clearly visible as strongly chitenized brown claspers and penis.

b. FEMALE the external genitalia are not conspicuous.

(3) Sex comb. Another criterion is the presence (male) or absence (female) of the sex comb, a group of heavy black bristles on the first tarsal member of the front foot. Under low magnification, the sex comb appears as an intense black speck. Ventral views are best suited for inspection; turn the individual until you are sure of the presence or absence of the sex comb. This criterion serves as a reliable check in doubtful cases, but this structure is too small to be useful in ordinary routine."

The original mating pairs with which I started my work were of two strains - pure white eyed and pure wild or red eyed. The original flies which were sent from the botanical supply house were destroyed because there was no way of differentiating between virgin and non virgin females. From the hatchings I received, I separated the males and females (there is no mating until at least 48 hours after hatching) of each type and placed them in separate bottles, grouped and labelled as to type and sex, so that I would have males and virgin females of both white and red types at any time for

further matings. The separating of the flies in this way will save a great deal of time for anyone who may wish to follow up this work; if he keeps his virgin females separated in this way, he will always have some on hand to make new crosses.

The first crosses made were between red males-white females, and white males-red females. Three pairs were introduced into each breeding bottle and left for 7 days at which time they were removed and destroyed, that they might not become mixed with the first filial generation. Larvae were distinguished in the breeding bottles, 3-4 days after the mating pairs were introduced. At 8-9 days, pupae appeared on the strips of paper toweling, and the first young flies hatched 12 days after the matings were made. Later experiments showed that hatchings can be expected as early as 10 days after mating, and as late as 17 days.

RESULTS: A - Parent generation.

I From 9 pairs of flies - pure white eyed female - red eyed male matings - - three pairs to a bottle

Bottle "A"	28 flies	9 white eyed males 19 red eyed females
Bottle "B"	15 flies	6 white eyed males 9 red eyed females
Bottle "C"	13 flies	4 white eyed males 8 red eyed females 1 white eyed female

(Note) The appearance of the white eyed female in Bottle "C" cannot be accounted for by any Mendelian laws. The only explanation I can offer is that it may be a mutant. Unfortunately this

fly died before it could be cross-bred to determine its genetic arrangement. One possibility for the appearance of this seemingly out of place type may be explained by the results which R. Goldschmidt obtained while working with Florida stock Drosophila; he found that phenotypic copies of later mutants appeared in early generations long before the mutation occurred. This white eyed female fly may have been a phenotypic precursor of a mutation to occur many generations later. There is a possibility of a mutant chromosome causing all mutations in other chromosomes, or mass-mutation may be a result of small chromatin rearrangements (gene mutations) caused by pre-existing chromosome rearrangements; so this fly may be the result of a gradual change.

II From 9 pairs of flies - pure red eyed female-white eyed male mating - - three pairs to a bottle

Bottle "D"	14 flies	7 red eyed males 7 red eyed females
Bottle "E"	16 flies	7 red eyed males 9 red eyed females
Bottle "F"	15 flies	7 red eyed males 8 red eyed females

B Mating F_1 Generation (Back crosses)

I 12 pairs of flies - impure red eyed female - red eyed male matings - 3 pairs to a bottle

Bottle A'	8 flies	2 red eyed males 2 white eyed males 4 red eyed females
Bottle B'	10 flies	3 red eyed males 2 white eyed males 5 red eyed females

Bottle C' 14 flies
4 red eyed males
3 white eyed males
7 red eyed females

Bottle D' 12 flies
3 red eyed males
4 white eyed males
5 red eyed females

II 12 pairs of flies - impure red eyed female-white eyed male.

Bottle E' 5 flies
1 red eyed male
1 white eyed male
1 white eyed female
2 red eyed female

Bottle F' 12 flies
4 red eyed males
1 white eyed female
2 white eyed males
5 red eyed females

Bottle G' 6 flies
1 red eyed male
1 white eyed male
2 white eyed females
2 red eyed females

Bottle H' 10 flies
3 red eyed males
2 white eyed males
2 white eyed females
3 red eyed females

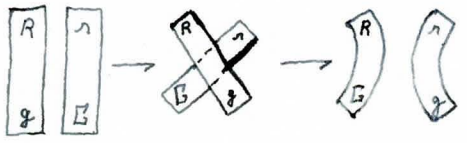
Time did not allow further matings, but all of the above results adhere closely to the results by both Mendel and Morgan. The fact that the number of white eyed flies does not quite agree with Mendelian counts, can be accounted for. White eye characteristic - a double recessive - is a lethal factor, and for this reason it can be expected that there will be fewer white eyed flies (especially females) hatching than red eyed flies. Small number of flies hatched will also effect number ratio of types.

Perfect charts for the breedings would be as follows:

A I_{p_1}	white eyed female (rr)	X	red eyed male (RY)
F_1	red eyed female (Rr)		1/2 of offspring
	white eyed male (rY)		1/2 of offspring
A II_{p_1}	red eyed female (RR)	X	white eyed male (rY)
F_1	red eyed female (Rr)		1/2 of offspring
	red eyed male (RY)		1/2 of offspring
B I_{p_1}	red eyed female (Rr)	X	red eyed male (RY)
F_1	red eyed females (RR)		1/4 of offspring
	red eyed females (Rr)		1/4 of offspring
	red eyed males (RY)		1/4 of offspring
	white eyed males (rY)		1/4 of offspring
B II_{p_1}	red eyed female (Rr)	X	white eyed male (rY)
F_1	red eyed females (Rr)		1/4 of offspring
	white eyed females (rr)		1/4 of offspring
	red eyed males (RY)		1/4 of offspring
	white eyed males (rY)		1/4 of offspring

Many attempts have been made to bring about mutations in Drosophila melanogaster. Irradiation and X-ray are two factors which are known to greatly increase the numbers of mutant forms. Beadle, Tatum and Clancy have found in their experiments that the V^+ hormone produced by fat bodies at the time of puparium formation, has an effect on production of eye pigments in these flies; they controlled the food supply of larvae of vermilion flies and established the fact that a low food level in the larval stage will cause an excess production of V^+ hormone in this type of fly and hence a change in eye pigmentation.

Mutations may occur from natural causes by the exchanging of loads (genes) by the chromosomes of the female parent in a dihybrid cross so that both dominant characteristics appear on the same chromosome, and recessive characteristics likewise; if eggs having this constitution are fertilized by male determining sperm, the resultant males would be double doiminant or double recessive for both characteristics. Morgan observed that this occurred in one case in a hundred.



If these chromosomes are fertilized by male determining sperm, the resultant males will appear to be out of line with Mendelian heredity. This is the result of a "crossover", and resulting types are mutations.

I made two attempts to bring about mutations by use of heat, but both attempts failed. However, I believe that a temperature of 32°-35° C will eventually result in mutant forms.

S U M M A R Y

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1. All recent work in the field of heredity can be traced to Mendel and Morgan.

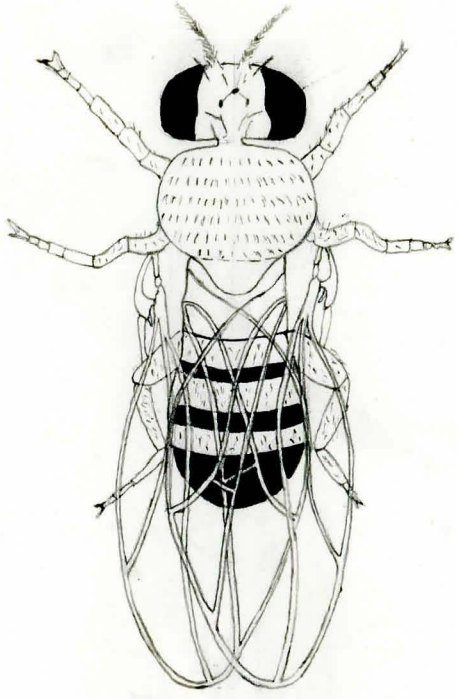
2. Proper culture media and sterile conditions are necessary for securing good hatchings of flies.

    a. The worker must keep a close watch for signs of mold, as this will kill his flies.

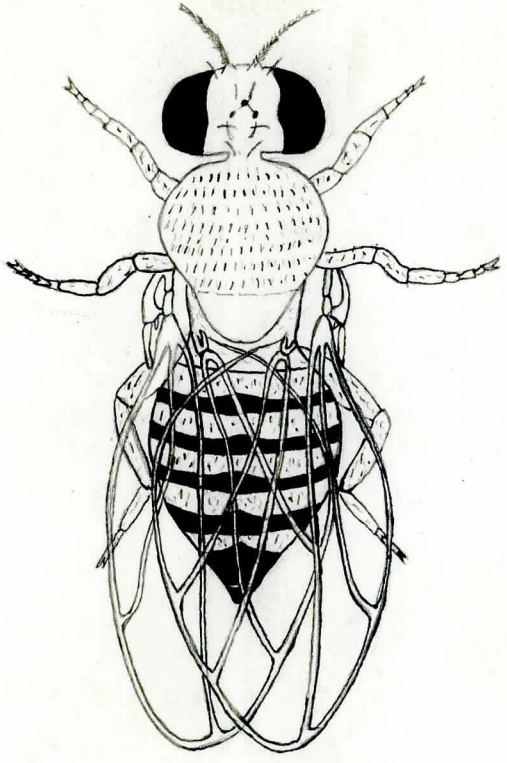
3. Young flies will hatch between 10 and 17 days after the eggs are laid. To secure full counts, the cultures should not be destroyed until at least 20 days after mating pairs are introduced.

4. The red eye characteristic is definitely a dominant, while white eye is definitely a recessive. These eye colors are proved to be sex-linked by the selective breedings which were carried out in this work.

5. Crossing over of genes on the chromosomes as well as many other natural phenomena such as heat, irradiation, etc. will cause mutations.



MALE



FEMALE

Drawing showing difference of sexes in Drosophila melanogaster.

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B I B L I O G R A P H Y

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