THE INFLUENCE OF LIGHT ON GENE FREQUENCY CHANGES IN LABORATORY POPULATIONS OF EBONY AND NON-EBONY DROSOPHILA MELANOGASTER

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THAT the mating behavior of ebony and non-ebony *Drosophila melanogaster* is influenced by light has been shown by RENDEL (1951) using the multiple choice method and JACOBS (1960) using the direct observation method. Both these authors found ebony flies to show greater sexual activity in the dark than in the light, while with non-ebony the converse was true.

Laboratory population studies in which ebony competes with non-ebony have shown ebony to decrease in frequency to about ten percent and then to stabilize (L'HERITIER, NEEFS and TEISSIER 1937). In similar studies, KALMUS (1945) found ebony to increase in numbers when the temperature and relative humidity were decreased from 25°C and 70 percent relative humidity to 12–15°C and ten percent relative humidity. The above authors postulated heterosis theories to explain stabilization of ebony following decline from an initial high frequency.

The present study was undertaken with ebony and non-ebony from a wild population to determine whether light influences the frequency of ebony in laboratory populations and also whether the multiple choice method would show a difference in mating success between ebony, non-ebony, and the heterozygote under different lighting conditions.

MATERIALS AND METHODS

The flies used were ebony (*e/e*) and light tan (+/+) collected from a wild population at Beaufort, North Carolina (JACOBS 1960). In an attempt to randomize genes away from the ebony locus so that the effects studied would be those differentially due to the *e* gene and its + allele, a series of crosses was continuously made. In the following description of these crosses, an asterisk indicates that the flies were grown at 25°C until pupation, at which time the pupae were placed at 15°C to cause development of a darker color of +/*e* as compared with +/+ to facilitate selection of +/+ for pure cultures. In all other cases, the flies

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were cultured at 25°C. This series of crosses is as follows, with females on the left:

P_1 $e/e \times +/+$
P_2 $+/e \times +/e$
*F_2 $+/+$ $+/e$ e/e
P_3 $+/+$ and e/e selected for pure cultures
$+/e \times +/e$
*F_3 $+/+$ $+/e$ e/e
P_4 Repeat as for P_3 and add $+/+$ and e/e cultures to those previously selected as soon as homozygosity is proved.

Homozygosity was proved by crossing numerous males from the presumed $+/+$ cultures $\times$ e/e females. This procedure has been continuously repeated as for P_3 and later generations for over three years to the date of the present investigation.

To obtain flies for study, $+/+$ males and females were placed in a five-liter wide mouthed egg-laying jar with a cloth sleeve at the opening through which petri dishes with medium were entered. In a similar egg-laying jar e/e males and females were placed, and in another, e/e females and $+/+$ males.

From the petri dishes, a block of medium with about 200 eggs and/or newly hatched larvae was cut. This was placed in a half-pint milk bottle with about 50 ml of medium. The cultures were incubated at 25°C in the dark. All medium used was of a modified Carpenter-Baker formula (JACOBS 1960). Populations were cultured in five-liter wide mouthed jars. A hole about 95 mm in diameter was cut into the lid and cardboard lid sealer, and a wire gauze was placed between the lid and sealer to make the jars fly tight. Newly emerged e/e adults, 100 of each sex, and five $+/+$ males were placed in each jar. Each day, the closed end of the jar was faced toward the light, and a 25 $\times$ 95 mm vial with about 10 ml of medium was added quickly to prevent escape of flies. The number of vials each jar contained at any one time throughout the study varied from about 20 to 40.

At about biweekly intervals, 500 flies were counted from each jar after the adults had been etherized to a very early immobile state by means of ether-soaked cotton in a finger bowl placed over the wire gauze opening as the jars lay horizontally. The larvae appeared little affected by the ether and remained mobile. The vials were then removed, and the nonstuck flies in the vials and jar were dumped through a funnel into a half-pint bottle. After the jar and gauze were cleaned, the adults, and vials which still contained larvae were readmitted. Two jars were kept in the dark in a 0.098 m^3 incubator, and two were kept in a 0.075 m^3 glass-paneled incubator, one above the other, 16 cm from a vertical B & L 500 ft-c microscope lamp containing two four-watt daylight fluorescent tubes 11 cm long. All jars were incubated horizontally. On the floor of each incubator was kept 1188 cm^3 of water in trays. Both incubators were kept at approximately 25°C with Fenwall No. 17500 thermoregulators with inherent sensitivity specification

of 0.1°F. The results of the population studies were drawn up at the end of a seven month ten day period after inoculation of the jars.

Multiple choice studies were made using etherized adults which had just emerged from the pupa cases and were still transparent, but which had expanded wings. Two types of crosses were made as follows: (1) ten *e/e* females × (ten +/+ males and ten +/*e* males) (2) ten *e/e* females × (ten +/+ males and ten *e/e* males). Each cross was repeated four times in the light and four times in the dark, giving a total of 16 separate crosses. Each cross was made in a half-pint milk bottle with 50 ml of medium sprinkled with about 50 mg of Fleischmann's dry active yeast. The +/*e* males had been grown from eggs of *e/e* females crossed with +/+ males. The crosses in the dark were made in the dark incubator described above; while the crosses in the light were made in the light incubator 16 cm in front of the fluorescent light where the population jars had been kept.

RESULTS

In all population jars, the frequency of *e/e* dropped from the initial 97.56 percent, and it dropped most rapidly in the light (Figure 1). In the seven month ten day period in the dark, the frequency of *e/e* reached a low of 7.02 percent in one jar and 6.47 percent in the other, while in the light it reached 4.76 percent in one jar and 3.47 percent in the other.

In the multiple choice tests in which *e/e* and +/+ males were placed with *e/e* females (where random mating would yield 50 percent *e/e* progeny) 22.53 percent of the progeny were *e/e* in the lighted bottles, and 60.20 percent were *e/e* in the dark bottles. This indicated a mating advantage of +/+ males in the light and *e/e* males in the dark (Table 1). The deviation from the expected 50 percent *e/e* in both conditions gave chi-square values of over 100 which is highly significant. In those bottles containing +/+ and +/*e* males with the *e/e* females (where random mating would yield 25 percent *e/e* progeny) there were 32.55 percent *e/e* progeny in the lighted bottles and 38.22 percent in the dark bottles. This indicated that +/*e* males had a mating advantage over +/+ males in light and

TABLE 1

Comparative maitng success of melanogaster *males as indicated by the percent of ebony progeny they produced when ten males of each genotype were introduced, in various competitive combinations, into culture bottles with ten ebony females at 25°C*

Competitive combination		Progeny produced in light			Progeny produced in dark		
		Ebony	Non-ebony	Percent ebony	Ebony	Non-ebony	Percent ebony
+/+ and *e/e* males	Observed	720.00	2475.00	22.53	1561.00	1032.00	60.20
	Expected	1597.50	1597.50	50.00	1296.50	1296.50	50.00
	Chi-square		964.00			107.92	
+/+ and +/*e* males	Observed	1351.00	2799.00	32.55	1477.00	2387.00	38.22
	Expected	1037.50	3097.50	25.00	966.00	2898.00	25.00
	Chi-square		126.31			360.41	

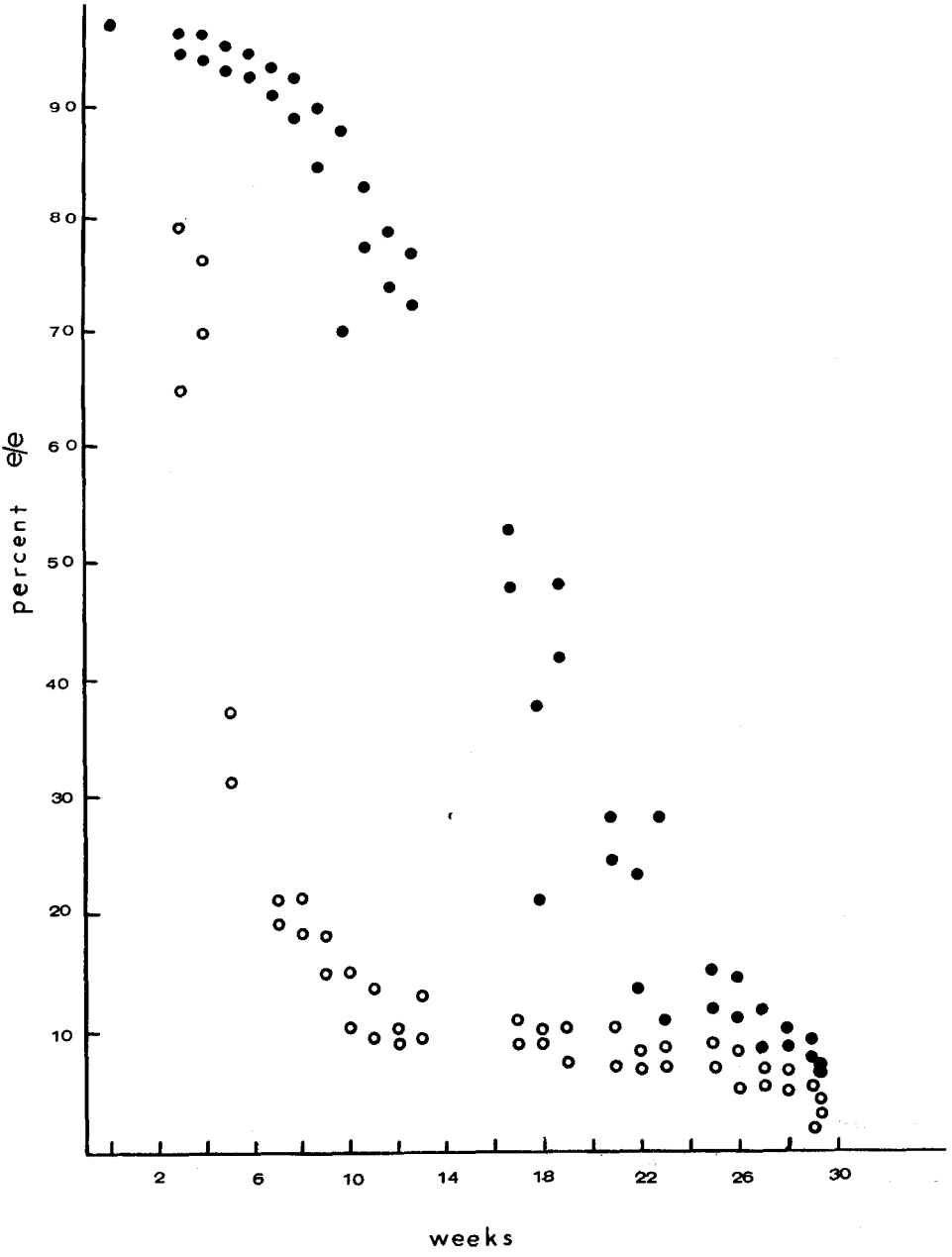

FIGURE 1.—Frequency of *e/e Drosophila melanogaster* in population jars after various intervals. Two jars were kept in dark conditions (solid circles) and two in lighted conditions (hollow circles). Each jar had been inoculated with 100 *e/e* males and 100 *e/e* females and five +/+ males.

dark, the differences from the expected 25 percent here again gave chi-square values of over 100. The +/*e* males in competition with the +/+ males had a greater mating advantage in the dark (to produce the 38.22 percent ebony progeny) than in the light (to produce the 32.55 percent ebony progeny). The chi-square value for this difference is 28.18 which is below the one percent probability level, and highly significant.

The question arose as to whether, perhaps, the above effect of "light" may really be due to light-heat raising the temperature of the flies by absorption. To test this, two bottles containing the *e/e* female and (+/+ and *e/e*) male combinations were placed, in the dark, at 22°C in the refrigerated incubator and two were placed in the dark, at 27°C in the ordinary incubator. The bottles were treated as before to tell whether the *e/e* males would still have an advantage over the +/+ males in these dark conditions with varying temperature.

The results showed no significant difference at the two temperatures. In the 22° bottles 1624/2498 or 65.01 percent of the progeny were *e/e*, while in the 27° bottles 1329/2120 or 62.68 percent of the progeny were *e/e*. The chi-square value for this difference is only 2.69, which is above the 30 percent probability level and not significant. Even if the difference would have been significant, it would not indicate that the "light" effect is really a heat effect, for if the ebony males were placed at a disadvantage in the light because of the heat, there should be more *e/e* progeny observed in the colder bottles instead of the fewer that were observed.

The mating advantage of +/*e* over +/+ males was supported by studies using the direct observation method described by JACOBS (1960). Males were isolated from females upon emergence and aged four days before being placed in observation cells. In each of two cells were placed 30 *e/e* females. In one of the cells were placed 30 +/*e* males, and in the other were placed 30 +/+ males. The cells were placed under 66.20 ft-c of daylight fluorescent light, and matings were counted for five hours. This procedure was repeated 16 times, in which period the +/*e* males mated 279 times while the +/+ males mated only 102 times, showing that, even in the light, the +/*e* males outmated the +/+ males. Previous studies under these conditions (JACOBS 1960) had shown that under the same lighting conditions +/+ males outmated *e/e* males in the same cells. Similar results using similar methods have been obtained by ELENS (1957).

DISCUSSION

The more rapid decrease of *e/e* in lighted than in dark population jars is probably due, at least in part, to the mating advantage afforded the +/+ males in light as compared with darkness, thereby allowing relatively greater numbers of +/*e* and *e/e* males to mate in dark conditions. This conclusion is supported by the multiple choice studies and previous direct observation studies in which it was found that +/+ flies mate and court more frequently in light than in diminished light (JACOBS 1960).

The tendency for the *e* gene to decline in population jars may be due to lessened

viability of ebony as compared with non-ebony under crowded conditions. MOREE (1952) found a steady decline in percent of ebony emerging from bottles as the number of $+/e$ parents was increased from two to 300, at the latter density of which ebony was reduced to 15.25 percent. The stabilization of the *e* gene in spite of negative selection against it, as found by L'HERITIER, NEEFS, and TEISSIER (1937), could be due partly to heterozygote mating superiority as shown by the multiple choice and direct observation studies. ELENS (1958) has also found that e^{11} in the heterozygous state increases sexual activity of males, and in another study (1957) he found e^{11} homozygotes to have decreased sexual activity as compared with the wild type.

If ebony stabilizes in population cages in competition with non-ebony, the question of its rarity in nature arises. In a study of a Beaufort wild population (JACOBS 1960) ebony was found only once. However, other dark forms intermediate between ebony and the light tan wild type were found commonly. These dark flies, when crossed with ebony, gave cultures ranging from ebony to dark without the appearance of a light tan form in subculture. The dark form appeared to be produced by an *e* allele with effects intermediate between *e* and + when homozygous. Such "mild" ebony alleles have also been reported from Texas (MARY ALEXANDER, personal communication). It may be that the frequency of *e* in wild populations in spite of $+/e$ superiority may be due partly to the interaction of other alleles complicating the population dynamics. Also other factors may alter the frequency of the *e* gene in nature. KALMUS (1945) has found evidence that temperature and humidity alter the frequency of ebony in laboratory populations.

In other Drosophila species dark forms persist in nature. FREIRE-MAIA (1949) has found a case of melanistic polymorphism in *montium*, and WARD (1952) has found eastern woodland forms of *melanica* to be darker than western desert forms. A similar situation appears in the dragonfly *Perithemis tenera* (JACOBS 1955). Studies in physiological genetics on these forms may help unravel the problem of melanistic adaptation in nature.

SUMMARY

Studies of laboratory populations of *Drosophila melanogaster* showed ebony, in competition with non-ebony, to decrease from an initial high frequency more rapidly in the light than in the dark. Multiple choice studies in which e/e competed with $+/+$ showed $+/+$ to have a mating advantage in the light, while e/e had a mating advantage in the dark. $+/e$ males showed a mating advantage over $+/+$ in light, and still more in the dark. Direct observation studies showed $+/e$ males to mate with higher frequency than $+/+$ males. The bearing of these results on population dynamics is discussed.

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LITERATURE CITED

ELENS, A. A., 1957 Importance selective des differences d'activite entre males ebony et sauvage, dans les populations artificialles de *Drosophila melanogaster*. Experientia **13**: 293–297.

1958 Le role de l'heterosis dans la competition entre ebony et son allele normal. Experientia **14**: 274–278.

FREIRE-MAIA, NEWTON, 1949 Balanced polymorphism in *Drosophila montium*. Evolution **3**: 98.

JACOBS, M. E., 1955 Studies on territorialism and sexual selection in dragonflies. Ecology **36**: 566–586.

1960 Influence of light on mating of *Drosophila melanogaster*. Ecology **41**: 182–188.

KALMUS, H., 1945 Adaptive and selective responses of a population of *Drosophila melanogaster* containing *e* and *e+* to differences in temperature, humidity, and selection for developmental speed. J. Genet. **47**: 58–63.

L'HERITIER, PH., Y. NEEFS, and G. TEISSIER, 1937 Apterisme des insects et selection naturelle. Compt. rend. (Paris) **204**: 907–909.

MOREE, RAY., 1952 Experimental measurement of the relative viability of the mutant "ebony" in *Drosophila melanogaster*. Am. Naturalist **86**: 45–48.

RENDEL, J. M., 1951 Mating of ebony, vestigial, and wild-type *Drosophila melanogaster* in light and dark. Evolution **5**: 226–230.

WARD, C. L., 1952 Chromosome variation in *Drosophila melanica*. Univ. Texas Publ. **5204**: 237–257.