NATURAL SELECTION AGAINST THE SEX-LINKED GENE FOR YELLOW BODY COLOR IN DROSOPHILA MELANOGASTER

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NATURAL SELECTION AGAINST THE SEX-LINKED GENE FOR YELLOW BODY COLOR IN *DROSOPHILA MELANOGASTER*

By

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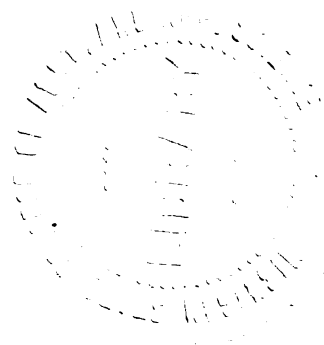

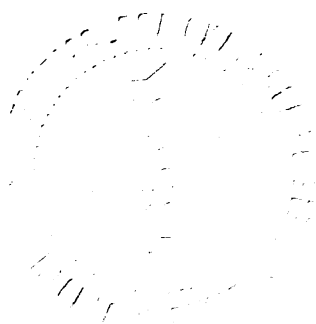

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INTRODUCTION

The experimental study of selection is necessary for an understanding of the process of evolution. Certain factors may modify the effect of selection against a recessive gene. Replacements of the loss of this gene may occur by migration or mutation. Gradual acquisition of modifier genes may lessen the intensity of selection. Superiority of heterozygous forms can offset selection pressure against homozygotes. If these phenomena occur at constant rates, then a state of equilibrium may occur, in which the gene frequency is fixed. Equilibrium for autosomal factors is reached in the first generation following random breeding, providing there is no selection pressure (Pearson, 1904). Refinements of this principle by Hardy (1908) assumed equal fertility, random mating, and independence from sex. A sex-linked gene is not independent from sex, however, since the female possesses a heterozygote class and the male does not. But Hogben (1946) and Li (1948) have shown that Hardy's Law of gene frequencies ($p^2 + 2pq + q^2 = 1$, Hardy, 1908) still applies at equilibrium in the case of sex-linked genes in the female.

The proportion of hemizygous males showing the sex-linked recessive trait is theoretically the square root of the proportion of females with the same trait:

Sex	male		female		
Genotype	Y	y	YY	Yy	yy
Frequency	p	q	p^2	2pq	q^2

This equilibrium for sex-linked traits is not reached in one generation of rendom breeding. If the proportion of dominant males in the initial population is equal to the proportion of dominant gametes among female gametes then under random breeding these proportions are fixed (Robbins, 1918). In mathematical terms, equilibrium occurs in the first generation after random breeding only if

$$p = p^2 + pq,$$
$$q = q^2 + pq. \qquad \text{or}$$

For all cases in which the gene frequency in the males is not equal to the gene frequency in the females, equilibrium is approached in an oscillatory manner (Li, 1948). According to Li, approximate equilibrium is attained in about fifteen generations. The difference,(d), between the gene frequency in each sex is halved in each generation,

$$d_n = (-1/2)^n d_0 \qquad \text{(Li, 1948).}$$

Thus, the difference between the frequencies of a recessive sex-linked gene in the males and females of any generation (d_n) is a function of that difference in the initial genera-

tion (d_o). In Hogbens' terminology,

$$d_n = (RY_o + RY_1)(-1/2)^n$$

(Hogben, 1946)

That is, the difference between the frequencies of a recessive sex-linked gene as between the males and females of any generation (d_n) is a function of the frequencies of this gene in the first and second generation males. After three or four generations, the mean values of any two consecutive frequencies are so close to their limiting values that equilibrium can be assumed (Hogben, 1946).

All these mathematical ideas assume no selection. It was suggested by Dr. Hunt that the effect of selection on a sex-linked trait could be tested in actual populations of Drosophila. Thus in this experiment with Drosophila melanogaster, the wild-type fly was introduced into each of two cages containing populations of recessive flies. Since the recessive allele is usually less advantageous to the species than is the dominant (Boyd, 1953), it was suspected that the frequency of recessive flies would decrease in each generation as a consequence of selection. Therefore, a differential food supply was employed in which one cage received approximately half as much food as the other cage. The generations were kept separate in order to measure the generation-to-generation frequency changes.

REVIEW OF LITERATURE

Equilibrium has been demonstrated in Drosophila by many researches. Investigations of equilibrium conditions have shown that sometimes the heterozygous form is superior to the homozygotes. Ingenious experiments (Dobzhansky, 1947) with chromosome inversions in D. pseudoobscura have demonstrated that, although the types of eggs laid are in proportions agreeing with Hardy's Law, the inversion heterozygotes are more frequent than expected. The adaptive superiority of the heterozygote thus protects both types of chromosomes from selective pressure and results in a balanced polymorphism. In Drosophila, nearly all inversion heterozygotes are adaptively superior to their corresponding homozygotes (Dobzhansky, 1951). Thus equilibrium conditions have been attained in many laboratory experiments.

An analysis of the color pattern in D. polymorpha (da Cunha, 1949) showed equilibrium due to a differential mortality between the egg and larval stages which favored the heterozygote. Dubinin and Tiniakov (1946) found heterozygote superiority in D. funebris to be the main cause of equilibrium. Similar results were reported by L'Heritier and Teissier (1933) and L'Heritier (1937) in D. melanogaster, by Reed and Reed (1948) in D. melanogaster, by Freire-Maia (1949) in D. montium, by da Cunha, Burla, and Dobzhansky (1950) in D. willistoni,

and by Shell (1952) in D. melanogaster.

Quite often such heterozygote superiority is accompanied by stronger selection pressures against one of the alleles than against the other. Thus elimination of such an unfavored gene may occur. Drosophila laboratories are constantly beset by contamination of mutant stocks by their wild alleles. Such contamination often leads to elimination of the mutant gene. The gene for vestigial wings, for example, may disappear rapidly when competing against its wild allele for normal wings (Spencer, 1932).

A sex-linked recessive gene would enjoy even less protection by the heterozygous state, since the males are never heterozygous. The low incidence of mutant genes in the sex chromosomes of wild-caught Drosophila, as compared with those in the autosomes, is evidence of this (Huxley, 1943). Thus the sex-linked gene, yellow, was strongly selected against in populations of D. melanogaster under optimum food conditions (Ludwin, 1951). Reed and Reed (1950) found that the sex-linked gene for white eyes in D. melanogaster decreased from a frequency of .500 to zero in twenty-five generations. Their excellent work showed selective mating to be the primary cause. Equilibrium was not attained due to failure of white males to breed as often as wild males. Neither of these latter two experiments with sex-linked genes involved a generation-by-generation frequency count.

The methods used by these investigators have been various. Most approaches were attempts to simulate nature, wherein

generations interbreed. These approaches utilized population containers which supplied fresh medium for the flies a desired time intervals. Pioneer work on cages of this type was performed by L'Heritier and Teissier (1933) and L'Heritier (1937). Later modifications (Dobzhansky, 1947) improved the efficiency of these cages. Dobzhansky's cage featured a detachable glass top on a wooden box with glass or screen sides. Fifteen holes in the bottom served to furnish a continuous supply of fresh food and to remove larvae and pupae for examination. A different technique was employed by Reed and Reed (1948, 1950). Two interconnected bottles formed the population unit. Examination was accomplished by etherization, removal, classification, and replacement of the "groggy" flies. Although these small bottle-units yielded fewer flies, the use of many units permitted random sampling. Variations of this two-bottle unit were used by da Cunha (1949) and Ludwin (1951). Cages and units of these types may allow approximation to free-living populations, but certain disadvantages also exist. Fly excreta may accumulate, mites may infest the population, or excessive dryness may occur (Wright and Dobzhansky, 1946).

For generation-to-generation frequency counts, a different type of cage is desirable. Fly populations in such a cage are killed and classified after laying the eggs which would develop into the flies of the next generation. These eggs are removed in order to clean the cages. Then the eggs and fresh food are replaced. This technique removes possibility of mite infestation and excess excreta accumulation. Shell (1952)

used glass-sided cages of this type for generation-to-generation studies of the gene for sepia eye in D. melanogaster. Similar cages were employed in this experiment.

METHODOLOGY

Materials

All operations were undertaken in the constant temperature room of the Natural Science Building at Michigan State College. The temperature was kept at 79° ± 1° F.(26° C.). Two cages with identical dimensions were employed to obtain population data. Each cage measured thirteen inches high, eighteen inches wide, and twenty-six inches long. Glass panes held by a frame of one-inch wood stripping formed the sides and top of each cage. Access to the interior was provided by sliding the front pane up its grooved stripping. Each cage had a plywood base, three-fourths inches thick, thirty-two inches long, and twenty-four inches wide.

The food of the flies was contained in Petrie dishes. The food consisted of a medium with the following ingredients:

10	liters of water
110	grams of agar
8	grams of Moldex
1/2	lb. baker's yeast (dissolved in water)
350	cc. of unsulphured molasses
350	cc. of Karo
1000	grams of corn meal

This medium was stored in Petrie dishes in the refrigeration room at 44° F. A strip of sterile paper toweling was laid on the surface of the medium in each Petrie dish to provide a place for larvae to pupate. Twenty-four hours prior to actual

insertion in a cage, the Petrie dishes were removed from refrigeration. This procedure permitted initiation of yeast growth and brought the medium to room temperature.

The sex-linked gene for yellow body color was chosen for use in this experiment. This gene has occurred spontaneously in D. melanogaster numerous times (Morgan, Bridges, and Sturtevant, 1925). It is at locus 0 of the X chromosome. A similar gene exists in the X chromosome in D. simulans, D. virilis, D. obscura, and D. willistoni. In D. melanogaster, the yellow phenotype is easily distinguished from the wild phenotype. The body color is rich yellow, while the hairs and bristles are brown with yellow tips. The hairs and veins of the wings are yellow. The phenotype is also classifiable in the larval stage. Larval setae and mouth parts are yellow to brown in color (Bridges and Brehme, 1944).

Procedures

The line of flies using relatively more food has been termed "Abundance"; the line using relatively less, "Scarcity". The only differences in the procedure between the two experiments were a slight variation between initial frequencies of the yellow gene and the number of dishes of medium used for each generation.

The "Abundance" line was started in half-pint milk bottles and transferred to its cage after the P_1 parents were removed. This P_1 generation comprised five wild males, forty-

seven yellow males, and forty-nine yellow females placed in a half-pint milk bottle and allowed to breed. After the appearance of the F_1 pupae, these P_1 parents were removed. They were classified as to body color and sex, then counted. The bottle containing the F_1 pupae was placed in the "Abundance" cage with three Petrie dishes. The food dishes were placed equidistant from the pupae bottle and from each other. A folded paper barrier was placed between the larva-pupa bottle to prevent larvae from crawling to the food dishes. The emerging F_1 flies bred and fed in the three food dishes. Some breeding occurred in the larva-pupa dish, also.

F_1 imagoes were permitted breed for seven days. They were then removed in order to prevent intermixture of two successive generations. Two days before the F_2 generation emerged, one dish of F_2 larvae and pupae was removed to be used in producing the F_2 imagoes. The larvae and pupae in the remaining dishes and the bottle, along with the F_1 parents were etherized and removed. These dishes and the bottle were discarded. The F_1 parents were classified and counted. The cage was cleaned and aired, then the one F_2 larva-pupa dish was replaced. Three Petrie dishes containing fresh medium were added as above. Thus the parents of each generation were classified apart from their offspring. This cage technique was repeated three generations after the appearance of the last yellow fly.

The "Scarcity" procedure was carried out in similar fashion. The P_1 generation consisted of four wild males, one wild female, one hundred and twenty-five yellow males, and ninety yellow fe-

males mated in a half-pint milk bottle as with "Abundance". The inclusion of the wild female was due to an error of classification, and occasioned the future use of the binocular scope for rechecking classes. The experiment was not invalidated, however, since the initial gene composition was known in both lines. After the appearance of the F_1 pupae, these P_1 parents were removed and classified. The larva-pupa bottle was placed in the "Scarcity" cage. Only one dish of food was added. F_1 flies emerging from the bottle bred and fed in this dish. After seven days, this F_2 larva-pupa dish was removed to be used in producing the F_2 generation. The F_1 parents were etherized and classified, and the cage was cleaned. The F_2 larva-pupa dish was returned along with one dish of food. This procedure was repeated until after three generations following the appearance of the last yellow fly.

Techniques

Removal of flies and dishes was accomplished in the following manner. First the cage's front pane was lifted several inches. One side of the opening was covered with a piece of cardboard to prevent escapes, while a dish of ether was quickly inserted. One Petrie dish containing the larvae and pupae of the next generation was removed. The pane was closed and sealed with tape to increase etherization efficiency. When the flies were completely etherized, the remaining dishes were removed and examined for imbedded flies. The flies on the

paper-covered floor of the cage were then swept onto a paper and removed. They were placed in a one half-pint bottle until examination.

The cage was then cleaned with Bon Ami and 70 percent alcohol, and a new floor of sterilized paper was provided. The previously-removed Petrie dish containing the eggs and larvae producing the next generation was replaced. Dishes of fresh food were added. Special care was given to prevent contamination at this point by stray flies in the room. The pane was carefully closed, and the cage was placed in its rack. After both cages had been emptied and restocked in this manner, the dead flies were classified and counted.

For classification the dead flies were removed from the container bottles and separated into classes on white paper in natural light. Classification should be performed immediately, since delay brings about shrinkage and deformation of the flies due to dessication. The yellow body color may be difficult to distinguish in immature flies, but the yellow wings are easily seen in all stages after eclosion. The flies were separated by the naked eye according to sex and body color. Then all four classes were re-examined by passing them under a binocular scope. Each class was then counted twice, and the numbers and percentages were recorded.

DATA

The unique gametic relations necessitated by sex-linkage can be analyzed by examination of the formulas developed by Li (1948). Let

p = dominant male frequency.
q = recessive male frequency.
r = homozygous dominant female frequency.
2s = heterozygous female frequency.
t = homozygous recessive female frequency.

Then,

$$r_n = p_n(p_{n-1}).....p^2$$

This means that the frequency of the dominant homozygous females of a given generation (r_n) is the product of the frequencies of the dominant males of that generation (p_n) and the previous one (p_{n-1}). Further, at equilibrium the frequency of the homozygous dominant females will be the square of the dominant male frequency (p^2).

The homozygous recessive female frequency of a given generation (t_n) is equal to the product of the frequencies of the recessive males of that generation (q_n) and the previous one (q_{n-1}). When equilibrium is reached the frequency of the recessive females is the square of the recessive male frequency (q^2). Thus,

$$t_n = q_n(q_{n-1}).....q^2$$

The frequency of the heterozygous females of a given generation ($2s_n$) is equal to the sum of the product of the dominant male frequency of that generation and the recessive male frequency of the previous generation plus the product of the frequencies of the recessive males of that generation and the dominant males of the previous generation:

$$2s_n = p_n(q_{n-1}) + q_n(p_{n-1}) \ldots . 2pq$$

In actual practice, the frequency of heterozygotes is found by calculating the frequency of both homozygotes and subtracting that from unity:

$$2s_n = 1 - (r_n + t_n)$$

The male frequencies show the typical oscillatory pattern that is masked by heterozygosity in the females. The frequency of either the dominant or the recessive males of a given generation is equal to the sum of half the frequency of the heterozygous females of the previous generation and the frequency of the respective homozygous females of the previous generation:

$$p_n = r_{n-1} + s_{n-1}$$

$$q_n = t_{n-1} + s_{n-1}$$

The male frequencies can also be calculated by other formulas:

$$p_n = 1/2(p_{n-1} + p_{n-2})$$

$$q_n = 1/2(q_{n-1} + q_{n-2})$$

(Hogben, 1946)

The oscillations in the frequencies of recessive males in successive generations are shown by Hogben's formula;

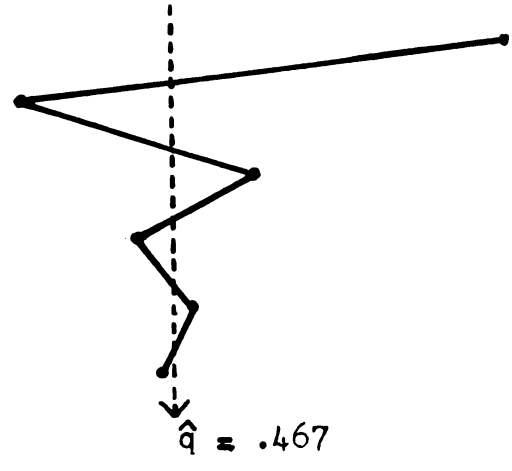
$$q_n = 1/3(q_o + 2q_1) + 2/3(q_o - q_1)(-1/2)^n$$

Table 1 shows an example in which the initial frequencies of the males have been arbitrarily selected.

TABLE 1

SAMPLE CASE OF SEX-LINKED EQUILIBRIUM FREQUENCIES

Generation	Frequency	.0 .1 .2 .3 .4 .5 .6 .7 .8 .9 1.0
q_o	1.0	
q_1	0.2	
q_2	0.6	
q_3	0.4	
q_4	0.5	
q_5	0.45	



The dominant male frequency is always unity minus the recessive male frequency. Both frequencies approach equilibrium values at the same rate then, and the equilibrium frequency for males can easily be calculated:

$$\hat{y} = q_o + 2/3\ (s_o + t_o) - q_o \qquad \text{(Li, 1948)}$$

The equilibrium frequency is equal to the sum of the original male frequency and two-thirds of the difference between the female and the male gene frequencies in the 0 generation. In the case of Table 1,

$$\hat{y} = 1.000 + 2/3\ (.200) - 1.000 = .467.$$

Application of this formula to this experiment shows that the equilibrium frequency of the yellow gene in the case of no mutation, no migration, and no selection would have been 0.968 in "Abundance" and 0.982 in "Scarcity". Table 2 shows the expected frequencies in "Abundance"; Table 3 shows those of "Scarcity". The actual gene frequencies, however, must be inferred from the observed phenotype frequencies. These are considered next.

Population Cage Data

Information from the population experiments has been completely presented in the form of tables and graphs for both cages and separately. Both "Abundance" and "Scarcity" showed frequency changes similar in direction but slightly different in intensity.

The frequency of yellow phenotypes in both sexes in "Abundance" dropped significantly (See Table 4.) in the generation 2. The corresponding increase in wild flies was accomplished solely by the reproductive ability of the heterozygous females in generation 1. Such a reproductive superiority indicated that the yellow gene might be eliminated in several generations. However, the yellow flies became more numerous each generation until they almost regained their original frequency by generation 6. Thereafter, a steady decrease of yellow occurred, until generation 18, when no yellow flies appeared. Since the three subsequent generations contained no yellow flies, it was assumed that the gene had been eliminated.

The initial erratic behavior of the yellow frequency may be due to certain uncontrolled environmental factors. Humidity changes occurred which may have affected the mutant genes. Also, food control varies due to different-sized populations living on the food (Reed and Reed, 1948). Such factors probably affected the flies showing the mutant trait more than the wild-types, since characters determined by mutant genes are more modifiable by environmental changes (Plunkett, 1932).

Table 5 shows a similar elimination of the yellow gene in "Scarcity". However, the loss of yellow was accomplished much more quickly than in "Abundance". The sharp decrease in yellow frequencies in both sexes in the second generation apparently occurred through lack of matings between yellow males and yellow females. This is indicated by the relative scarcity of yellow females in generation 2 (only 2.6 percent of the

total flies). As in "Abundance" however, a period of recovery occurred. The recovery peak was only half that of "Abundance". Generation 10 shows an abrupt elimination of yellow phenotypes in both sexes. Such abrupt elimination of detrimental alleles is one aspect of the "Hagedoorn effect". This phenomenon pertains to the behavior of detrimental allelomorphs in the low frequency ranges. Increased random deviations either eliminate the factor or throw it up into a higher frequency range where stronger selection pressures exist (Kemp, 1929). Thus the increased randomness of frequency changes in yellow phenotypes at low frequencies indicated a lower selection pressure in "Abundance" than in "Scarcity". "Scarcity" yellow phenotypes, however, were completely eliminated in both sexes, and from a higher frequency. This pointed to a differential selection pressure in the two populations.

Graphical comparison of the frequencies in "Abundance" and "Scarcity" illustrates the process of differential elimination of yellow phenotypes in each sex. Figure 1 compares the proportion of yellow females in each generation in both populations. It can be seen that similar changes took place in each generation until the sixth generation. At this point the "Scarcity" yellow female proportion dropped considerably while the "Abundance" increased to its highest peak. The yellow male proportions in both populations, as shown by Figure 2, show even more concordance up until generation 5. Such concordance may occur through extraneous environmental factors

simultaneously affecting both populations. For this reason the generations were staggered; the second generation in "Abundance" coincides in time with the first in "Scarcity". The initial dip and recovery in both populations may represent some factor inherent in gene competition.

We are unable to explain the abrupt rise in the frequencies of yellow flies from the fourth to the sixth generations of the "Abundance" experiment, and a similar rise from the third to the fourth generations of the "Scarcity" series. Therefore, to estimate the rates of selection pressure these initial increases in the proportions of yellow flies will not be considered.

Consider the proportion of yellow flies among the total flies as shown in Figure 5 and tabulated in Table 6. A comparison of elimination rates beginning at phenotype frequencies of similar magnitude should give a general picture of any differential selection pressure. Elimination in "Scarcity" occurred during five generations following the generation 5 frequency of yellow, 38.6 percent, while in "Abundance" complete elimination did not occur until nine generations after a yellow frequency of 34.6 percent in generation 9. In other words, the mean elimination rate from the initial selected frequency level in "Scarcity" was -7.72 percent per generation. The mean rate from a similar frequency level in "Abundance" was only -3.84 percent. Figure 5 illustrates the changes in the proportions of yellow flies in both populations. The right hand column in Table 6 gives the t values for the differences

in the percentages of yellow flies in the two populations. All t values over 1.96 can be considered statistically significant (Dixon and Massey, 1951).

In sex-linked equilibrium the square of the frequency of yellow males should theoretically equal the frequency of yellow females in the same generation. In Tables 7 and 8 the right-hand column compares these generation values. At low frequencies, the yellow female frequencies are consistently lower than expected. This occurred in both "Abundance" and "Scarcity". Differential mortality in the yellow females or mating discrimination against the yellow males could cause such a phenomenon.

Graphical representations of the frequencies in Tables 7 and 8 illustrate generations in which selection may have "relaxed". Generation frequency changes which are opposite in direction in each sex are typical of the approach towards equilibrium with non-adaptive sex-linked genes. Such sex-linked fluctuations should be more evident where selection had "relaxed". Perhaps this is the case in generations 3, 4, 5, and 14 in "Abundance" (Figure 3) and in generations 4, 5, and 8 in "Scarcity" (Figure 4). Food deficiencies in "Scarcity" may have prevented more such unmaskings of sex-linked fluctuations.

Assortative Mating

The following data strongly suggest the presence of assortative mating. This phenomenon has been reported in yellow flies by Tan (1946), Sturtevant (1929), Rendel (1945), and Merrell (1949). All these authors have reported a definite discrimination against yellow males on the part of both types of females.

Matings were devised to test these findings. Two types of tests were employed; male preference and female preference. In the male preference tests, five males of one type (yellow or wild) were placed with five yellow females and five wild females. The females were all virgins and about twenty-four hours old. All of the wild females in the experiment were heterozygous. After five hours together, the fifteen flies were removed, and each female was placed in a separate food bottle. A record was kept of the offspring of each fertilized female. Fertilized yellow females produced all yellow males, and fertilized heterozygous wild females produced half yellow and half wild males. Twenty-one successful matings were obtained in these male preference tests. Table 10 shows the results.

The second type of experiment tested female preference. Five virgin yellow females were placed with five yellow and five wild males. Also, five wild females(heterozygous) were placed with five yellow and five wild males. In the case of the yellow female preference tests, the phenotypes of the F_1

females indicated the successful male. If all the F_1 females were yellow, then the successful male was yellow; if all the F_1 females were wild, then the wild male was the successful fly; and if there occurred both wild and yellow F_1 females, then both male types had fertilized the female. Twenty-three yellow females were fertilized, and the wild males were successful in twenty-one.

In the case of the heterozygous wild female preference tests, the successful male was again indicated by the phenotypes of the F_1 females. If the F_1 females were all wild, then the wild male was the successful male; if half the F_1 females were yellow and half were wild, then the yellow male was successful. All twenty-four fertilized heterozygous females produced all wild F_1 females, which indicates that no yellow males had ever been successful. The results are shown in Table 9.

Similar results were obtained by Merrell (1949) with tests of yellow and wild preferences in D. melanogaster. Test results can be used to determine mate selection coefficients. Such coefficients in turn can be applied to equations which calculate expected gene frequencies. Mating coefficients have been worked out by Merrell (1950). The equations are as follows:

AA = number of yellow male→ yellow female matings.
AB = number of yellow male→ wild female matings.
BB = number of wild male→ wild female matings.
BA = number of wild male→ yellow female matings.

M_f = relative frequency of matings by females.
M_m = relative frequency of matings by males.

The coefficient of female mating is a ratio of fertilized yellow females to fertilized wild females,

$$M_f = \frac{AA + BA}{BB + AB}.$$

The coefficient of male mating is a ratio of successful yellow males to successful wild males.

$$M_m = \frac{AA + AB}{BB + BA}.$$

Applying the coefficients to the genotype equations, (pages 12-14)

$$q_n = \frac{s_{n-1} + M_f(t_{n-1})}{(1-t_{n-1})\ M_f(t_{n-1})}$$

$$p_n = 1-q_n$$

$$r_n = \frac{(p_{n-1})\ (p_n)}{p_{n-1} + M_m(q_{n-1})}$$

$$t_n = \frac{M_m(q_{n-1})(q_n)}{p_{n-1} + M_m(q_{n-1})}$$

$$2s_n = 1 - (r_n + t_n)$$

When these equations are in turn applied to population data, comparisons can be drawn concerning the selection pressure in the actual populations. The material in Tables 9 and 10 yield the following assortative mating coefficient values:

$$M_f = 1.1$$

$$M_m = 0.08$$

However, Merrell's work with the same gene involoved 437 fertilized females compared to only 68 in our experiment. Since the tests in this experiment do not contradict Merrell's results, the relative mating coefficients used for frequency calculations were derived from his tests:

$$M_f = 1.4$$

$$M_m = 0.1 \qquad \text{(Merrell, 1949)}$$

Both Merrell's coefficients have been incorporated into the formulas shown on the preceding page.

For comparative purposes these mating coefficients must be applied to generations which show little or no selection abatement. Thus, the last thirteen generations of "Abundance" have been chosen for comparison. When Merrell's coefficients are applied to the probable genotype frequencies of generations

6 to 18 in "Abundance", the resultant expected frequencies are as shown in Table 11. The irregular changes in "Scarcity" do not lend themselves to ready comparison with Merrell's coefficient-curve (Figure 6), but the general trend is similar. Thus assortative mating is one possible cause for the elimination of yellow genes, but the difference between "Abundance" and "Scarcity" is probably due to other factors relating to the food deficiency in "Scarcity". In view of this, tests were conducted in regards to differential viability or vigor between the egg and imago stages.

Viability

When virgin heterozygous females are mated with yellow males, four classes of offspring are expected. These four classes, grey males, yellow males, grey females, and yellow females, are expected in equal proportions. Any deviations from this 1:1:1:1 ratio might indicate a differential selection in the form of reduced vigor, increased mortality, or lengthened rate of development. Tests were made in half-pint milk bottles. The number of parents was varied in order to discover whether increased population pressure was a factor in the selection. No significant deviation from expected sex ratios was noted. Therefore, both sexes of yellow flies were included in a single class with an expected frequency of .500. Table 12 shows that in progeny from one female up to ten fe-

males no significant selection differential exists. The information concerning the number of progeny per female per day is similar to Pearl's findings (Pearl and Parker, 1922) concerning population density. Pearl concluded that fewer eggs were laid in dense populations (Pearl, 1927). If the density effect on egg production were differential, then selection could operate.

Density

The food deficiency in "Scarcity" was due to the fact that all the eggs of each parent generation were laid in one Petrie dish while the eggs of each "Abundance" parent generation were laid in three Petrie dishes of food, one of which was selected for the next generation. Thus the food dish in "Scarcity" fed more flies than the dish in "Abundance". Since all the Petrie dishes used in both cages contained approximately the same amount of food, then there was less food per fly in "Scarcity".

To check the effect of density, the total population was correlated with the decline of yellow frequency in each generation. The coefficient of correlation calculated from 28 generations was 0.531. This value exceeds the normal coefficient of correlation value for two independent variables when n (number of generations) = 28 at the 1% level of significance (p.164, Dixon and Massey, 1951). From this it can be

said that the frequency decline of yellow phenotypes is NOT independent of the density of the population. Therefore, population density is a factor in the elimination of the yellow gene. Thus the higher densities of the "Scarcity" generations correspond with the sharper decreases of the yellow frequency. The less dense populations of "Abundance" produced generations with relatively smaller decreases in the frequency of yellow. The mean population per generation for "Scarcity" was 1,438 flies, compared to 1,036 flies for "Abundance".

The rate of increase of the total population falls off as the density increases (Pearl, 1927). Kemp (1929) suggested that the decrease in offspring in dense populations is due to a smaller proportion of the population engaging in reproduction. The diminished breeding unit could have contained relatively more wild flies in the "Scarcity" generations of our experiment. However, Pearl held that decreased egg production caused this decrease in the number of progeny.

L'Heritier (1937) explained the phenomenon by larval mortality. If Pearl's explanation is the answer, then egg production could occur differentially. If L'Heritier's is the answer, then larval differential mortality could take place. This latter mode was not detected in the tests in our experiment, although these tests involved less than eleven pairs of parents in the largest mating. Density-produced phenotypic alterations in size, however, may be indicative of

corresponding physiological changes. The gamete production of smaller yellow flies may have dropped more than that of smaller wild flies. Mating discrimination against yellow males may have become more pronounced with the smaller yellow males.

Rate of Development

Although yellow larvae were noticed to be more sluggish than wild larvae, no observable differences in the rates of development occurred. On the other hand, Neel (1941) found that in starved larvae, eclosion took place at about the same time as in well-fed larvae and even slightly earlier at times. For our experiment, developmental time in the wild stock was compared with that of the mutant stock. The times for development for wild and yellow flies in competition with each other were also noted. Significant differences were not observed.

TABLE 2

EXPECTED PHENOTYPIC FREQUENCIES
IN "ABUNDANCE", NO SELECTION

	MALE %		FEMALE %	
Gen.	Wild	Yellow	Wild	Yellow
0	9.6	90.4	-	100.0
1	-	100.0	9.6	90.4
2	4.8	95.2	4.8	95.2
3	2.4	97.6	7.1	92.9
4	3.6	96.4	5.9	94.1
5	3.0	97.0	6.5	93.5
6	3.3	96.7	6.2	93.8
7	3.1	96.9	6.3	93.7
8	3.2	96.8	6.2	93.8
9	3.2	96.8	6.3	93.7
n	3.2	96.8	6.3	93.7

TABLE 3

EXPECTED PHENOTYPIC FREQUENCIES
IN "SCARCITY", NO SELECTION

	MALE %		FEMALE %	
Gen.	Wild	Yellow	Wild	Yellow
0	3.1	96.9	1.1	98.9
1	1.1	98.9	4.2	95.8
2	2.1	97.9	3.2	96.8
3	1.6	98.4	3.8	96.2
4	1.8	98.2	3.5	96.5
5	1.7	98.3	3.6	96.4
6	1.8	98.2	3.5	96.5
7	1.8	98.2	3.6	96.4
n	1.8	98.2	3.6	96.4

TABLE 4

NUMBER AND PROPORTION OF COLOR AND SEX PHENOTYPES AMONG TOTAL FLIES IN "ABUNDANCE"

	Male				Female				
	Wild		Yellow		Wild		Yellow		
Gen.	N	%	N	%	N	%	N	%	Total
0	5	.050	47	.465	0	.000	49	.485	101
1	0	.000	132	.426	39	.126	139	.448	310
2	262	.228	290	.252	336	.292	261	.227	1149
3	160	.307	134	.257	116	.222	112	.215	522
4	144	.113	491	.384	460	.360	184	.144	1279
5	103	.129	294	.367	170	.212	234	.292	801
6	30	.022	650	.476	118	.086	568	.416	1366
7	88	.089	384	.387	146	.147	373	.376	991
8	167	.186	309	.344	227	.253	194	.216	897
9	277	.220	349	.277	548	.435	87	.069	1261
10	228	.256	210	.236	420	.471	33	.037	891
11	368	.340	173	.160	510	.472	30	.028	1081
12	756	.422	211	.118	822	.458	4	.002	1793
13	596	.454	73	.056	640	.487	5	.004	1314
14	715	.435	107	.065	821	.500	0	.000	1643
15	269	.440	8	.013	334	.547	0	.000	611
16	706	.461	22	.014	805	.525	0	.000	1533
17	526	.468	3	.003	595	.529	0	.000	1124
18	734	.498	0	.000	741	.502	0	.000	1475
19	694	.503	0	.000	687	.497	0	.000	1381
20	502	.505	0	.000	492	.495	0	.000	994

TABLE 5

NUMBER AND PROPORTION OF COLOR AND SEX PHENOTYPES AMONG TOTAL FLIES IN "SCARCITY"

	Male				Female				
	Wild		Yellow		Wild		Yellow		
Gen.	N	Prop.	N	Prop.	N	Prop.	N	Prop.	Total
0	4	.018	125	.568	1	.005	90	.409	220
1	31	.022	641	.464	32	.023	676	.490	1380
2	149	.259	139	.234	277	.481	15	.026	576
3	279	.267	278	.266	305	.292	184	.176	1046
4	235	.138	639	.374	724	.424	109	.064	1707
5	198	.242	202	.247	304	.372	113	.138	817
6	351	.311	186	.165	534	.473	59	.052	1130
7	245	.228	271	.253	496	.462	61	.057	1073
8	406	.244	374	.224	726	.436	160	.096	1666
9	1154	.347	466	.140	1626	.489	79	.024	3325
10	645	.488	0	.000	677	.512	0	.000	1322
11	921	.510	0	.000	885	.490	0	.000	1806
12	1167	.531	0	.000	1031	.469	0	.000	2198
13	1364	.489	0	.000	1425	.511	0	.000	2789

TABLE 6

PROPORTION OF YELLOW FLIES AMONG TOTAL FLIES: "ABUNDANCE" AND "SCARCITY"

Gen.	"Abundance"	"Scarcity"	t Value
0	.950	.977	1.1
1	.874	.954	4.0
2	.480	.260	9.8
3	.471	.442	1.1
4	.528	.438	5.0
5	.659	.386	11.4
6	.892	.217	45.0
7	.764	.309	23.3
8	.561	.321	12.0
9	.346	.164	13.3
10	.273	.000	18.2
11	.188	.000	15.7
12	.120	.000	15.8
13	.059	.000	9.1
14	.065	.000	10.8
15	.013	.000	2.6
16	.014	.000	4.7
17	.003	.000	1.88
18	.000	.000	.000
19	.000	.000	.000
20	.000	.000	.000

TABLE 7

PROPORTION OF YELLOW FLIES IN EACH SEX: "ABUNDANCE"

	Male Proportion		Female Proportion	
Gen.	Wild	Yellow	Wild	Yellow
0	.096	.904	.000	1.000
1	.000	1.000	.219	.781
2	.475	.525	.563	.437
3	.544	.456	.509	.491
4	.227	.773	.714	.286
5	.259	.741	.421	.579
6	.044	.956	.172	.828
7	.186	.814	.281	.719
8	.351	.649	.539	.461
9	.442	.558	.863	.137
10	.521	.479	.927	.073
11	.680	.320	.944	.056
12	.782	.218	.995	.005
13	.891	.109	.992	.008
14	.870	.130	1.000	.000
15	.971	.029	1.000	.000
16	.970	.030	1.000	.000
17	.994	.006	1.000	.000
18	1.000	.000	1.000	.000
19	1.000	.000	1.000	.000
20	1.000	.000	1.000	.000

TABLE 8

PROPORTION OF YELLOW FLIES IN EACH SEX:
"SCARCITY"

	Male Proportion		Female Proportion	
Gen.	Wild	Yellow	Wild	Yellow
0	.031	.969	.011	.989
1	.046	.954	.045	.955
2	.525	.475	.949	.051
3	.501	.499	.624	.376
4	.269	.731	.869	.131
5	.495	.505	.729	.271
6	.654	.346	.901	.099
7	.475	.525	.890	.110
8	.521	.479	.819	.181
9	.712	.288	.954	.046
10	1.000	.000	1.000	.000
11	1.000	.000	1.000	.000
12	1.000	.000	1.000	.000
13	1.000	.000	1.000	.000

TABLE 9

ASSORTATIVE MATING: FEMALE PREFERENCE

Female Genotype	Total Females Fertilized	Successful Males			
		Yellow		Wild	
		N	%	N	%
yy	23	2	8.7	21	91.3
Yy	24	0	0.0	24	100.0

TABLE 10

ASSORTATIVE MATING: MALE PREFERENCE

Male Genotype	Total Males Successful	Fertilized Females			
		Yellow		Wild	
		N	%	N	%
y	9	5	55.6	4	44.4
Y	12	6	50.0	6	50.0

TABLE 11

EXPECTED FREQUENCIES OF GENOTYPES: "ABUNDANCE", FROM MERRELL'S COEFFICIENTS

	Male		Female			
Gen.	Wild (p)	Yellow (q)	Wild (r)	Heterozygous (2s)	Yellow (t)	Total Yellow
6	.044	.956	.002	.170	.828	.892
7	.065	.935	.020	.344	.636	.778
8	.153	.847	.063	.441	.496	.682
9	.236	.764	.151	.576	.273	.516
10	.396	.604	.298	.555	.147	.373
11	.543	.457	.471	.470	.059	.258
12	.689	.311	.635	.341	.024	.179
13	.797	.203	.763	.229	.008	.107
14	.874	.126	.853	.144	.003	.065
15	.924	.076	.911	.088	.001	.034
16	.955	.045	.940	.060	.000	.022
17	.970	.030	.965	.035	.000	.014
18	.982	.018	.979	.021	.000	.009

TABLE 12

VIABILITY RESULTS

Number of Female Parents	Seventh Day Yellow Offspring Total	Percentage of Yellow Offspring	X^2	Imagoes Female Day
1	144	46.2	0.289	20.6
1	160	53.5	0.245	22.9
2	185	49.0	0.200	13.2
3	178	54.4	0.387	8.5
4	224	50.4	0.003	8.0
5	268	43.8	0.769	7.7
6	227	42.7	1.066	5.4
7	169	42.4	1.155	3.4
8	309	49.4	0.007	5.5
9	278	47.3	0.146	4.4
10	304	50.0	0.000	4.3

FIGURE 1

FREQUENCY OF YELLOW FEMALES AMONG TOTAL FLIES

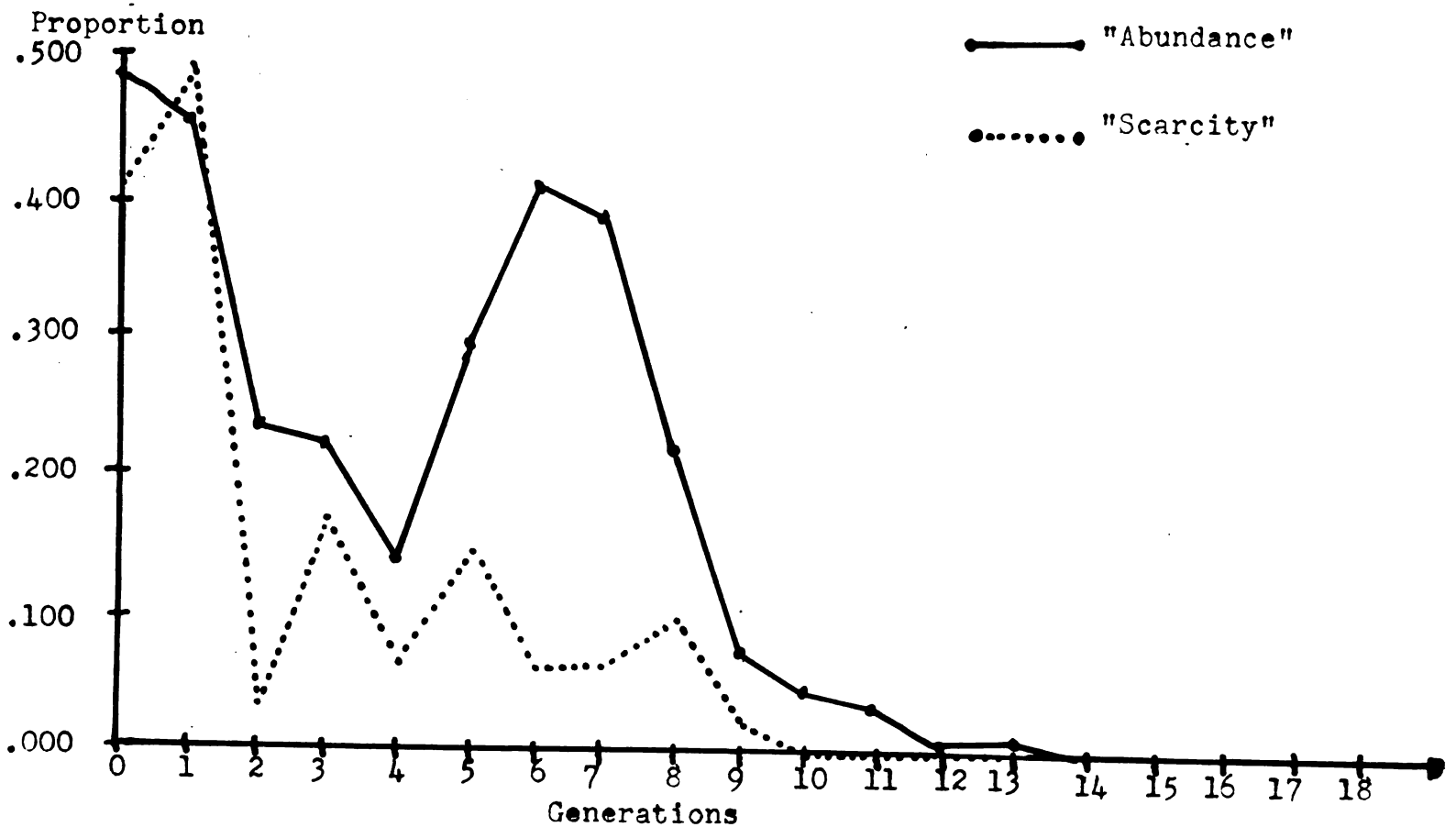


FIGURE 2

PROPORTION OF YELLOW MALES AMONG TOTAL FLIES

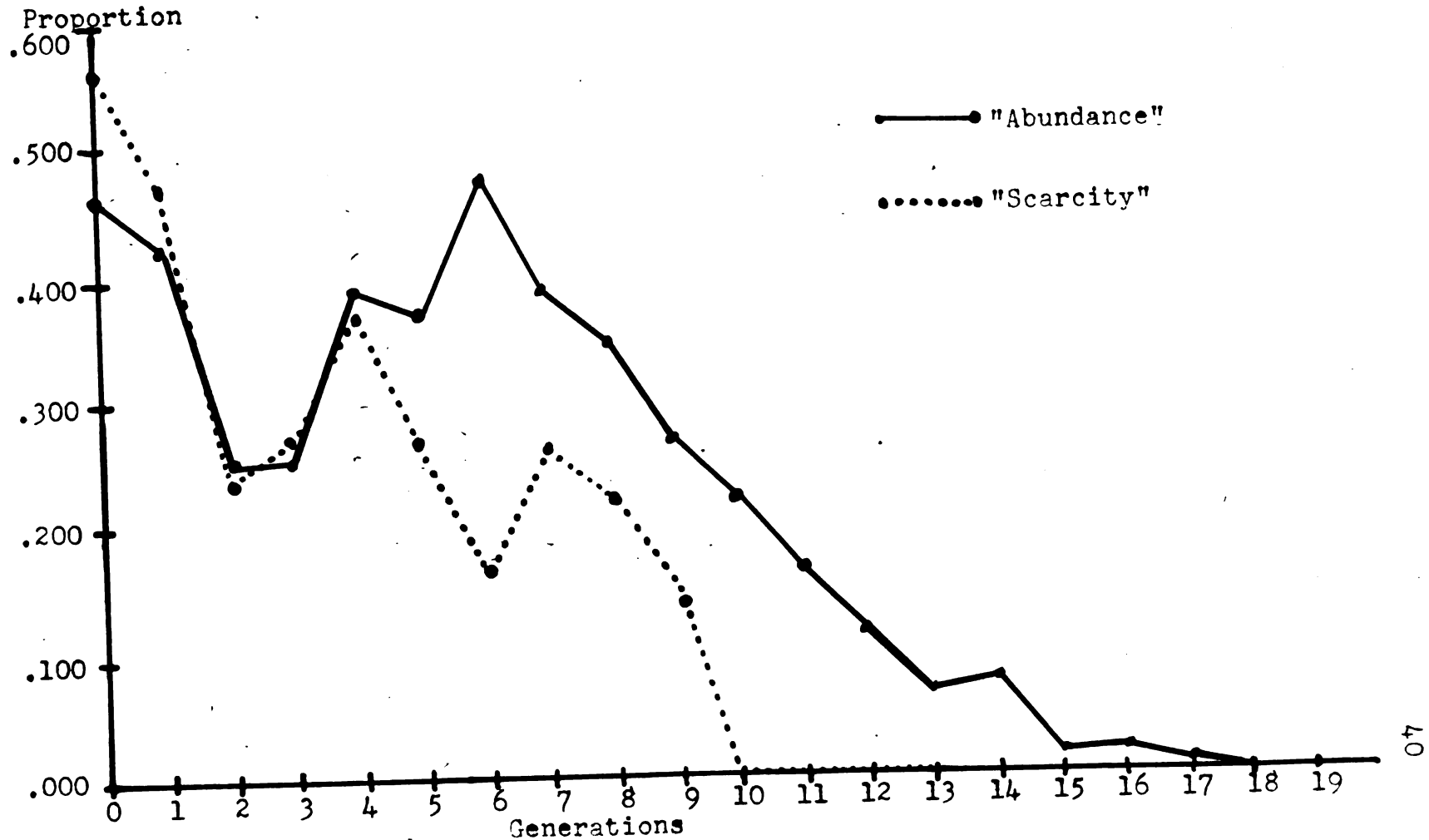


FIGURE 3

PROPORTION OF YELLOW FLIES IN EACH SEX, "ABUNDANCE"

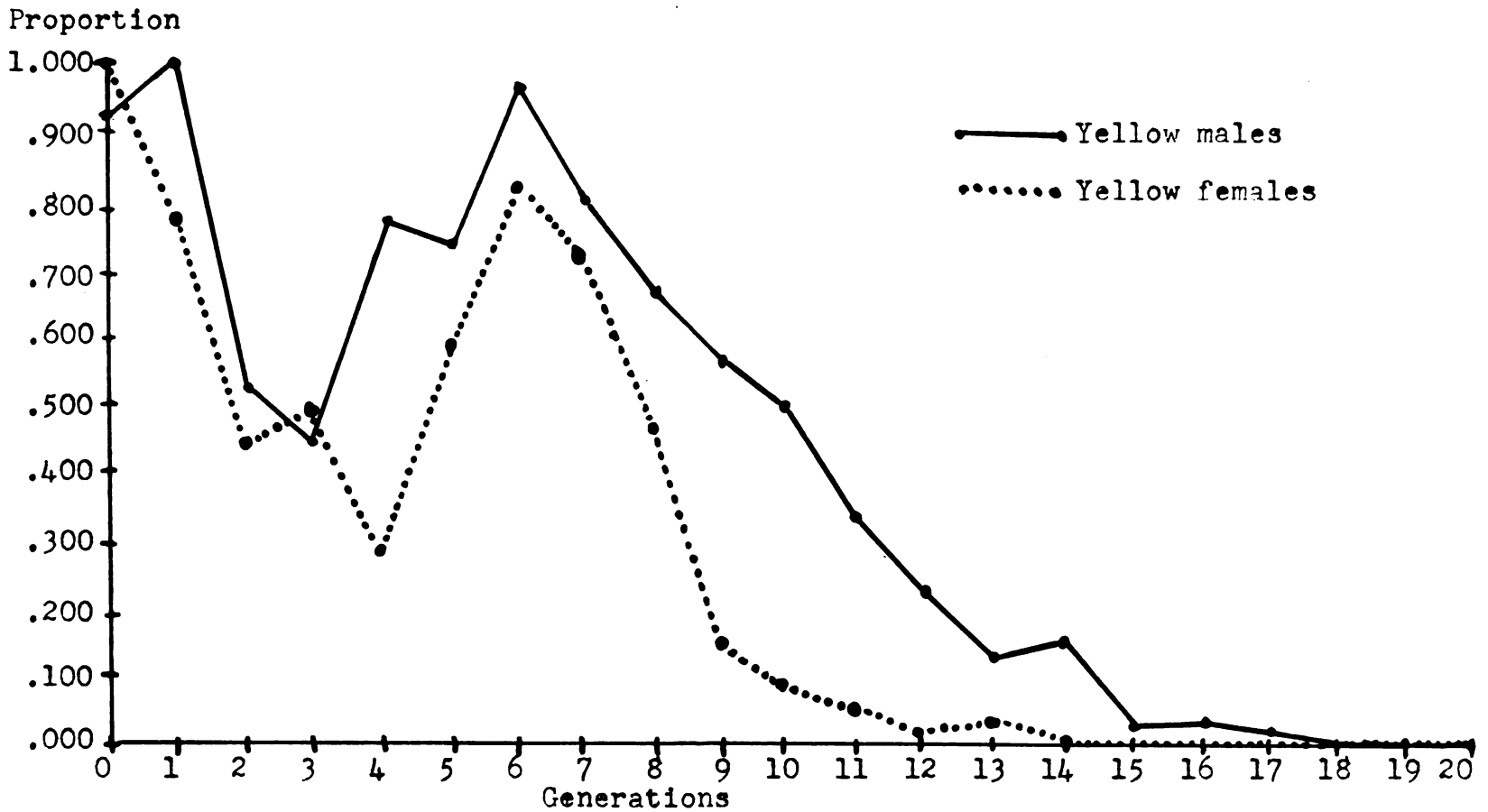


FIGURE 4

PROPORTION OF YELLOW FLIES IN EACH SEX, "SCARCITY"

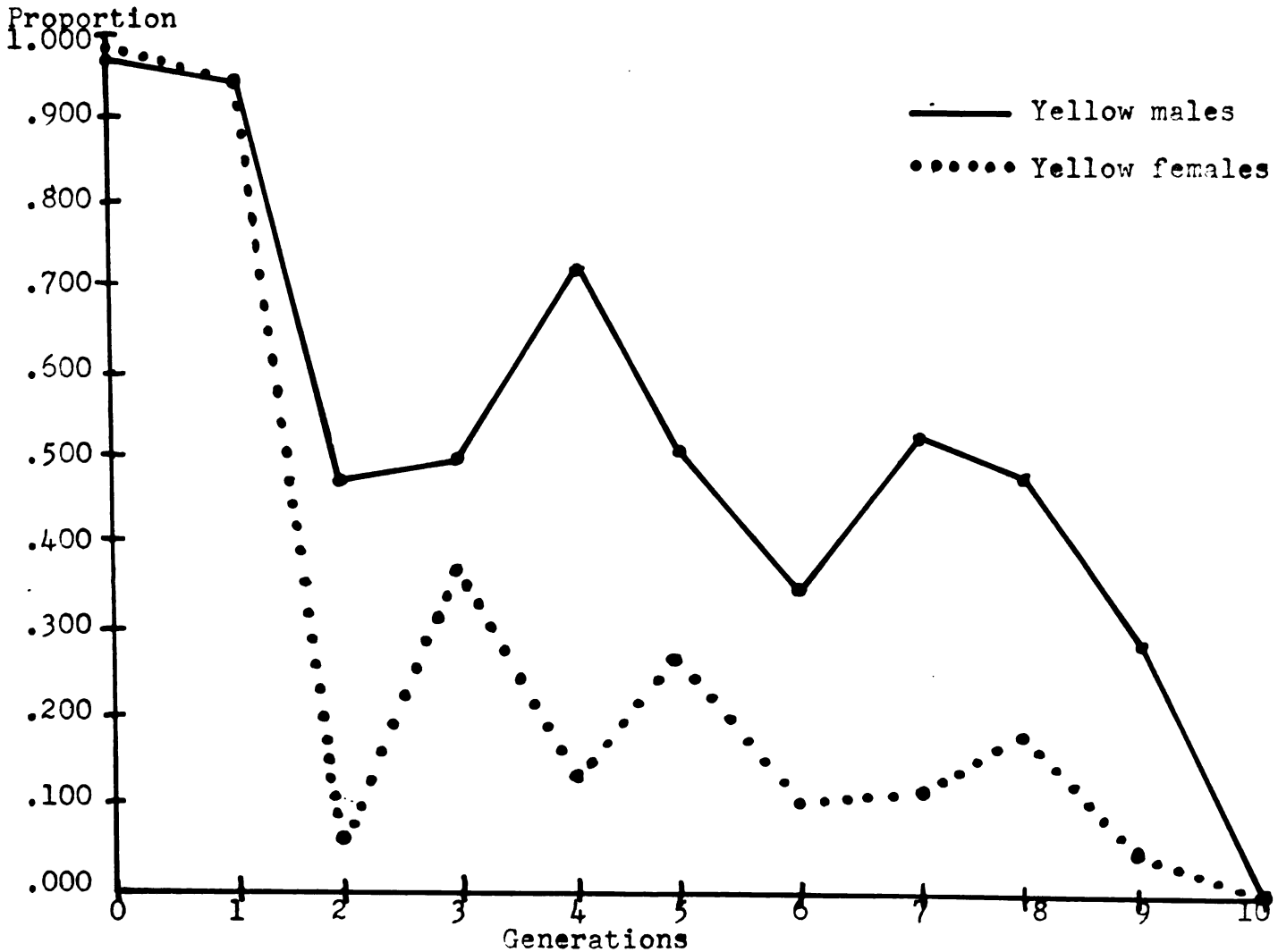


FIGURE 5

PROPORTION OF YELLOW FLIES AMONG TOTAL FLIES

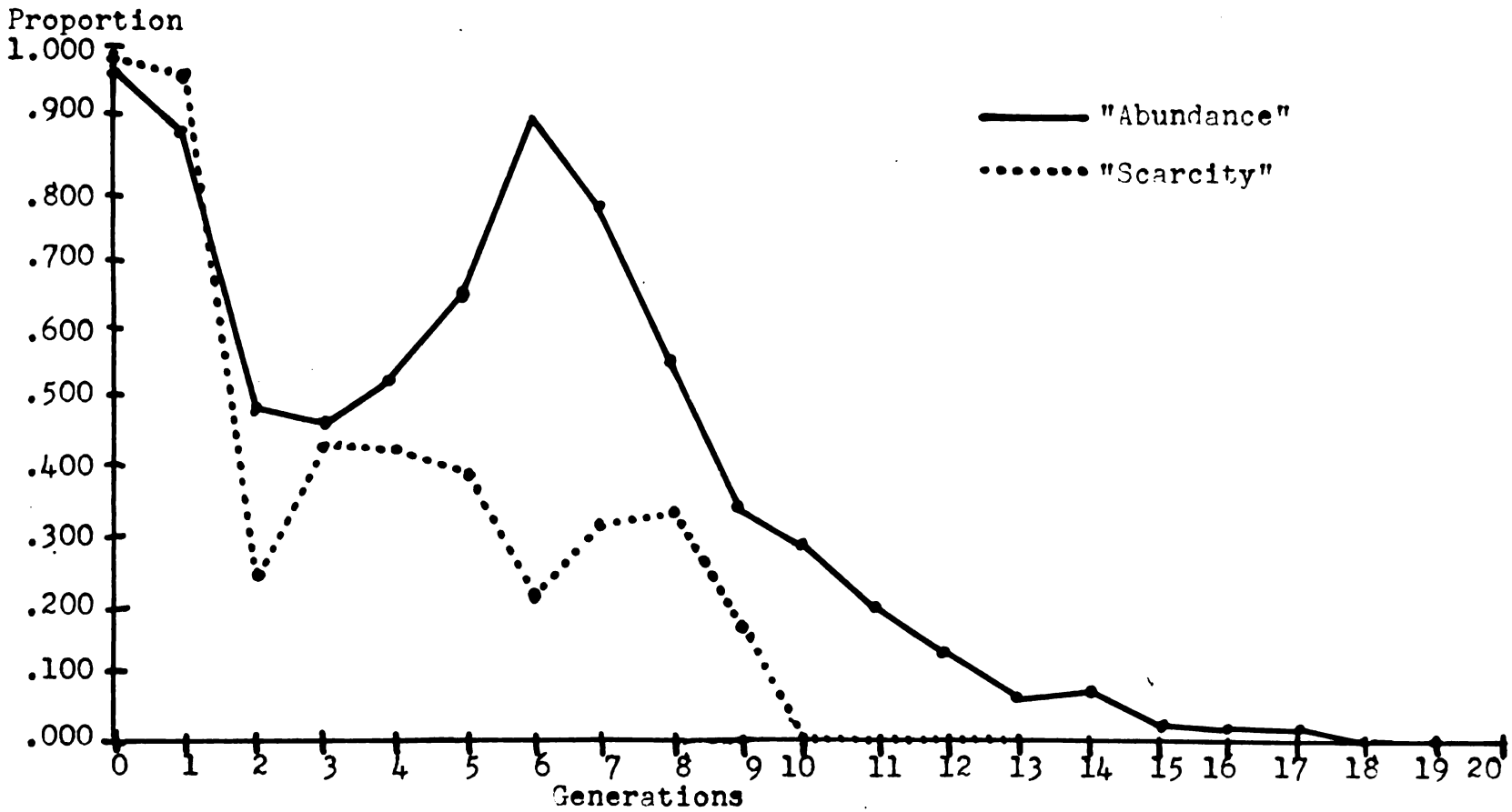
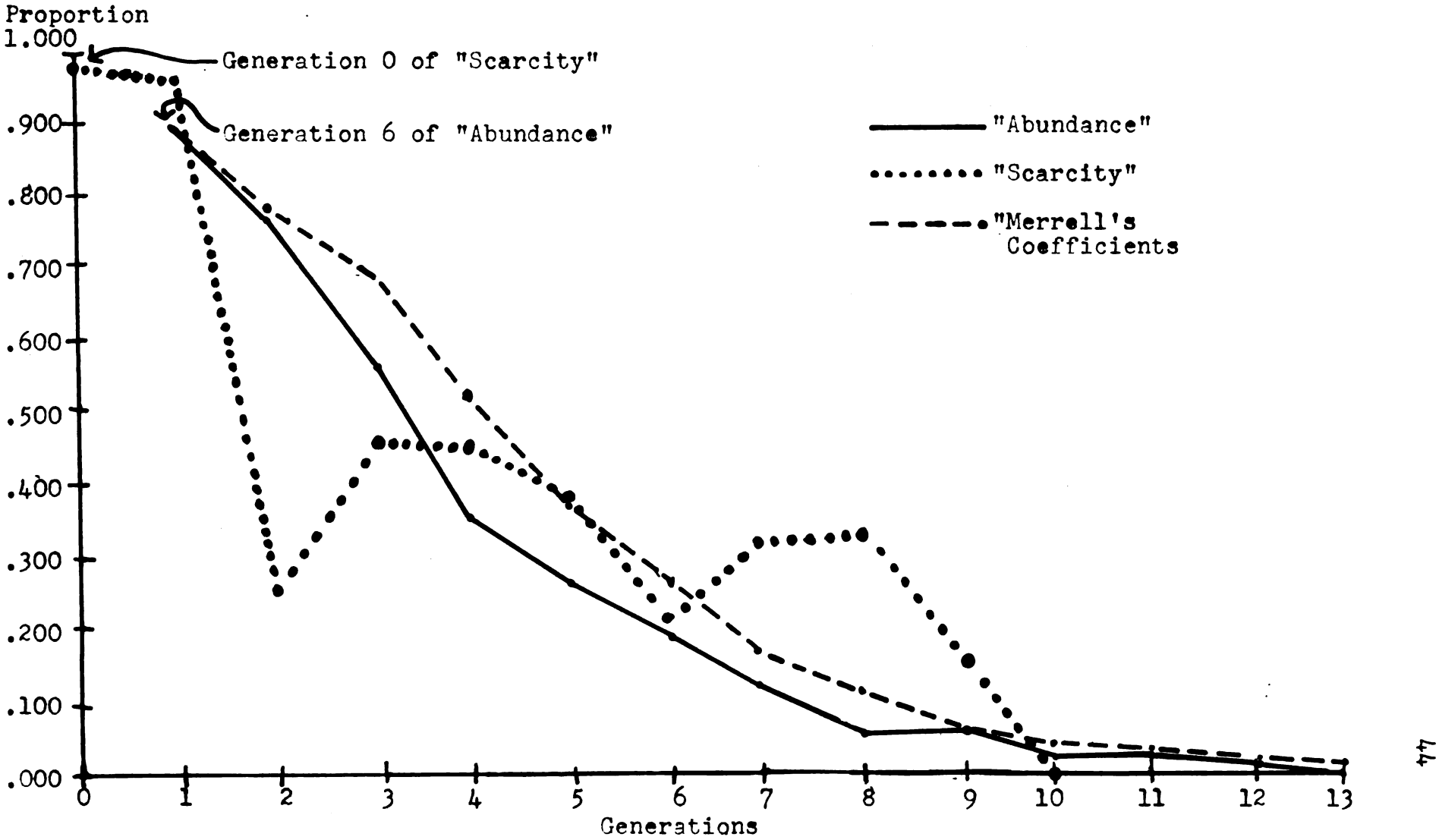


FIGURE 6

SELECTED FREQUENCY CURVES: TOTAL YELLOW AMONG TOTAL FLIES



DISCUSSION

In an article defending his theory of equilibrium, Hardy (1908) admitted that selective influences caused the entire situation to become "...greatly complicated...". Simpson (1951) warns that selection "...involves complex and delicate interplay with those genetic factors in populations...". Advice more pertinent to this problem was offered by Wright (1939) when he cautioned that with sex-linkage selection effects "...may be rather complicated because of the necessity of considering the sexes separately". Since no equilibrium was reached in either "Abundance" or "Scarcity", the manifold effects of selection had to be analyzed.

Types of Selection and Developmental Stages

Basically, selection is differential reproduction (Simpson, 1951). As such, selection mainly proceeds through a continuum of relative effectivity; from maximum potency to complete sterility, from highest viability to complete lethality, or from random copulation to absolute assortative mating. These differentials exist in three general forms; mortality, gamete contribution, and fertility. They may occur at different stages of development in every organism. In Drosophila, five main developmental stages can be considered with respect to selection: the adult, the gamete, the egg, the larval, and the pupal stages.

Mortality

The mortality of the adult fruit fly varies considerably. Spencer (1932) found that vestigial-winged flies lived longer than normal-winged flies when subjected to complete lack of food and water. Under optimum conditions the normal-winged flies lived longer. Differential adult mortality could not have been a factor in the elimination of yellow flies in this experiment. Less than 0.5 percent were found dead in the medium. In assortative mating tests, however, yellow males died significantly more often than any other type. This may have been caused by a differential viability with respect to ether.

There is no available evidence concerning differential mortality in gametes.

A differential mortality somewhere between the egg and larval stage was studied in D. polymorpha (da Cunha, 1949). This differential favored the heterozygote and resulted in equilibrium. There is no evidence of such a mortality differential with respect to the gene for yellow body color in D. melanogaster. Crosses in D. virilis between yellow males and heterozygous females yielded four classes in approximately equal proportions (Metz, 1916). The expected offspring of such a cross are males and females which are wild and yellow in a 1:1:1:1 ratio. The same results were obtained in this experiment (See Table 12) with D. melanogaster. These results suggest that no differential mortality exists in the gamete,

egg, larval, or pupal stage. This is a startling piece of evidence, considering that D. melanogaster lays ten times as many eggs as will develop (L'Heritier, 1937). Even in the absence of competition in optimum food conditions, only 43 percent of the eggs in one of L'Heritier's strains reached the adult stage. Apparently, such heavy selection pressure is random, in the light of the viability tests shown in Table 12. Perhaps the tests were not severe enough. A study should be done using the technique of J. V. Neel (1941). Neel studied crossing-over in starved larvae which had been fed only water-moistened Kleenex. Such severe starvation might introduce differential selection pressures which would be absent in the milder tests such as were carried out in this experiment.

Sturtevant (1929) mentioned that the viability of yellow phenotypes in D. simulans was "Usually good", and the gene for yellow in that species is allelomorphic with the same gene in D. melanogaster. Viability, as with other selective differentials, is remarkably sensitive to environmental change. For example, Timofeeff-Ressovsky (1933), found that the mutant, eversae, was superior to the wild fly at 24° - 25° C., but inferior at 15° - 16° C. and 28° - 30° C. No evidence of reduced viability was observed in our experiments, however.

Differential Gamete Contribution

A numerical disparity in gamete contribution can be caused by various factors. Assortative mating is often the primary

causative agent. Wright (1921) described two types of assortative mating based on somatic resemblance: perfect and imperfect. Had perfect assortative mating been the case here, then heterozygosity in the females would have rapidly diminished until only flies homozygous for yellow and for wild remained. The gene frequencies would have been unchanged, however.

Imperfect assortative mating, however, may mean partial failure of gene transmission on the part of a discriminated class. Such partial discrimination has been demonstrated by mating tests with sex-linked genes in D. pseudoobscura (Tan, 1946), D. subobscura (Rendel, 1945), and D. melanogaster (Rendel, 1945; Merrell, 1949; Reed and Reed, 1950). Tan showed that yellow females were preferred over wild females by both types of males. Merrell demonstrated the same phenomenon, but Rendel stated that yellow females mate normally. Tests in our experiment did not contradict the hypothesis that yellow females are preferred. Rendel (1951) wrote that it is not always possible to distinguish between a specific mating preference and an indirect effect of general vigor. In fact, Sturtevant (1929) concluded that the weaker and more inactive female is more likely to be mated with. However, weakening experiments did not increase the frequency of "cross-mating". This condition results in a biological paradox in that less-vigorous flies enjoy a reproductive advantage due to lack of vigor.

But in our experiment any mating superiority enjoyed by yellow females is more than cancelled by the strong discrimi-

nation against yellow males by females of both phenotypes. All mating tests with yellow have shown this fact. Merrell (1949), for instance, found that yellow males of D. melanogaster in competition with wild males mated successfully with yellow females only 11 percent of the time and only 6 percent with wild females. Such breeding discrimination against yellow males along with a preference for yellow females results in a relatively higher yellow male frequency in the offspring than expected. On the other hand, the class of yellow females is smaller than expected (See Tables 9 and 10).

Fertility

Hogben (1946) lists numerous criteria used to define fertility. The egg-laying capacity of the female, the viability of spermatozoa, the length of reproductive cycle, and the interval which elapses between two fertile periods are a few. The frequency of mating attempts would influence assortative mating tests. Our tests, however, reflect actual discriminations and not merely a lessened frequency of mating attempts. This conclusion is based on actual observations of rejections of yellow males by females. As for the above criteria of fertility, they were not tested in our experiment.

Rate of Development

Although no mutant gene is known that affects only the larval or pupal stage of D. melanogaster (Morgan, Bridges,

and Sturtevant, 1925), the yellow larvae are certainly physiologically different from the wild larvae. Whether their slower movements and limited range reflect lessened viability or slower growth rates is not known. Many mutants have excessively slow rates of development (Bridges and Brehme, 1944). Curly-wing in D. pseudoobscura, for instance, develops slower than the normal-wing (Dobzhansky and Spassky, 1944). No evidence for differential development rates was observed in our experiment, however.

Conclusions

As Sewall Wright (1921a) predicted, a differential reproductive rate among classes prevented equilibrium. Investigation of the selection modes causing this differential reproduction suggests that the main factor involved in the elimination of yellow was assortative mating. A search for the cause of the differential effect of density in "Scarcity" and "Abundance" was attempted. Adult mortality was ruled out. A survey of selection literature brought out several possible effects of population density: a decreased breeding unit, a decreased egg production, or an increased larval mortality. Several other possibilities can logically be considered: increased discrimination against yellow males or decreased developmental rates. The fact that one successful courtship stimulates other males to redoubled activity (Rendel, 1951) may be a factor. Any of these variables may have operated

in a differential manner, thus causing a differential elimination of yellow. These same factors may have varied with the frequency levels, with the humidity, or with any of numerous envoronmental effects.

SUMMARY

1. Selection prevented equilibrium between the sex-linked gene for yellow body color and its wild allele.

2. The main selective pressure was possibly due to assortative mating. Specifically, the major factor was probably severe discrimination against yellow males during courtship.

3. Frequency changes in yellow flies were also significantly correlated with population density. The greater population per generation in "Scarcity" indicated less food per fly than in "Abundance".

4. Evidence was presented to show that differential viability in the egg-to-imago stages was probably not a factor.

5. Differential mortality in the adults was probably not a factor.

6. Observations indicated that a differential rate of development was probably not a factor.

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