THE DECLINE IN THE FREQUENCIES OF EBONY, SCARLET, AND VESTIGIAL PHENOTYPES IN DROSOPHILA MELANOGASTER WHEN IN COMPETITION WITH "WILD TYPE"

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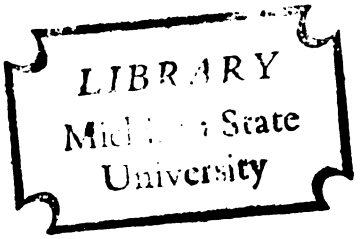
MASTER OF SCIENCE



THE DECLINE IN THE FREQUENCIES OF EBONY, SCARLET, AND VESTIGIAL PHENOTYPES IN DROSOPHILA MELANOGASTER WHEN IN COMPETITION WITH "WILD TYPE".

By

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INTRODUCTION

Population study, with respect to gene frequencies, is one of the main areas of investigations by geneticists. They want to know the probability of survival of a mutant gene in a population where there is competition between the different phenotypes and genotypes. They also want to know how the frequency of a gene can be affected by certain types of breeding, such as random mating, inbreeding, etc. These geneticists are interested, also, in the effects on gene frequency of such variable factors as temperature, humidity, population density, season, food, genetic drift, and above all, natural selection. Do these factors disturb the Hardy equilibrium? How do they operate to alter this theoretical equilibrium? At what stage of development of an organism is selection strongest? What are the survival or adaptive values of certain genes? What is the relation of changes in gene frequency to the evolution of a species? What genes and traits are favored by natural selection? How does mutation in a static or in a dynamic environment affect the genotype and phenotype of a species?

The investigations and writings of Dobzansky[8-15], the Reeds[46-8], Wright[63], Carson and Stalker[6,7], Spiess[50-53], Ludwin[41], Wallace[61], Merrel[43], and da Cunha[16,17], to mention a few, are noteworthy in connection with answers to such questions in genetics, as stated above.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Dobzansky[8] in Drosophila pseudoobscura and Dubinin and Tiniakov[20] in D. funebris found that populations of these fruit flies which live in different habitats, often differ in the relative gene frequencies of their gene arrangements. They also found that the composition of a single population may vary appreciably from season to season, and that the carriers of different gene arrangements may be favored or discriminated against by different environments. The seasonal changes in the compositions of the gene frequencies of Drosophila populations are adaptive responses of the living species to the succession of seasonal environment.

The Reeds[46] devised an experiment to demonstrate the superiority of heterozygotes to homozygotes in Drosophila. They stated that balanced polymorphism which resulted from the superiority of heterozygotes, preserved a few homozygotes of all types, thus permitting the species to react adaptively to the changes in the environment.

In his paper on the "Roles of Directed and Random Changes in Gene Frequency in the Genetics of Populations"[63], Sewall Wright concluded that fluctuations in some genes are undoubtedly governed by violently shifting conditions of selection for others in the same population. He stated

that accident of sampling and random drift due to sampling in small populations were important factors of gene fluctuations.

Spiess[50], in "Experimental Population of Drosophila persimilis From an Altitudinal Transect of the Sierra Nevada", found that a population cage containing WT and MD chromosomes, when placed in 25°C constant temperature room for two generations, showed no change in their gene frequency. But when these same chromosomes were transferred to 16°C, immediately a significant change in frequency took place. He found that WT increased nearly 20% with a drop in MD, over a period of three generations, and an equilibrium was reached at 58% WT and 42% MD.

Ludwin[41], in "Natural Selection in D. melanogaster Under Laboratory Conditions", found that genetic drift operates largely upon genes with low selective values whether the values are positive or negative.

In his article, "Modification of Adaptive Values of Chromosomal Types in D. pseudoobscura by Nutritional Variables", Antonio Brito da Cunha[17] showed that the type of food on which his fruit flies fed might be a very important factor in the distribution and in the relative gene arrangements in the natural population of this species. He found that the relative adaptive values of both homozygotes and heterozygotes showed heterosis. In others heterozygotes

were inferior to homozygotes. This showed that the genes carried in chromosomes with different gene arrangements made their carrier adaptive to different foods.

The investigations of these biologists, and others to be mentioned later, have revealed to us the causes of differential expressivity of mutant genes in many organisms, especially in Drosophila. They have added to man's understanding of the mechanism of organic evolution. The extinction of many animals and plants is also explainable on the basis of natural selection which favors organisms with inheritable adaptive traits. This inheritance is made possible only through the self reproducing entity, the gene.

The original purpose of this research was to test Hardy's law[29] which is fundamental in genetic study of populations. This law was formulated by G. H. Hardy in 1908 at Trinity College, Cambridge. Hardy apparently had read the proceedings of the Royal Academy of Medicine. He found that in these proceedings Yule had suggested, as a criticism of the Mendelian position, that if brachydactyly was dominant, "in course of time one would expect, in the absence of counteracting factors, to have three brachydactylous persons to one normal".

Hardy, refuting this criticism, said that if Aa is a pair of Mendelian characters, A being dominant, and that

in any given generation a gene, A, with a frequency of p and its allele a with a frequency of q (where p plus q = 1) would form the following proportions:

$$\begin{array}{cccc} AA & & Aa & aa \\ p^2 & + & 2pq & + & q^2 & = 1 \end{array}$$

These proportions agree with Ching Chun Li's[40] mathematical evaluation of this equilibrium law in his "Introduction to Population Genetics", and Lancelot Hogben's[34]. Hardy said that this population would be at equilibrium theoretically after a single generation of random breeding. He also hypothesized that if random mating continued the ratios of the three geneotypes would be the same in all and indefinite generations provided:

1. That the population is fairly large.
2. That mating is random.
3. That sexes are evenly distributed among the three varieties.
4. That all are equally fertile and viable, and
5. The two alleles are autosomal.

N. P. Dubinin and G. G. Tiniadov[20], working with D. funerbris, as mentioned above, observed that this type of equilibrium was established in accordance with Hardy's law. Sara Shell[55], who worked with sepia eye in D. melanogaster populations, stated, in her thesis for the M.S. degree in zoology, that the wild-sepia populations she studied did not follow the ratios expected by Hardy's law, but were modified by selection pressures.

Types of Drosophila Melanogaster Used

In the author's experiment Drosophila melanogaster was used. Its genetics is well understood, and its reproduction is fast, so that large populations were raised in a short time. Its period of development is short and it is a very convenient laboratory animal. Dr. H. R. Hunt, my adviser, suggested that since this fruit fly is so easy to raise and is genetically widely understood, it should be used, because these traits are easy to identify. Ebony refers to the blackish color of the body. The gene which is responsible for this color is on the third chromosome. It is completely recessive to its wild type allele. The scarlet eye is quite different from the red eye of the wild Drosophila. The gene for scarlet is on the second chromosome. It is completely recessive. It has complete penetrance[59]. One can differentiate the scarlet from the wild easily if the animals have just been killed. No microscope is necessary in the identification of the eye color, but occasionally a hand lens is useful.

The vestigial fly has wings which are abnormal in form, and much reduced in size. These insects cannot fly. The gene for vestigial is on the second chromosome. It is completely recessive. Genes on the first chromosome were not selected for these experiments, to avoid the complications of sex linkage and to satisfy one of the conditions specified in Hardy's Law.

Hardy's theory is applicable to both plants and animals, as long as the conditions inherent in it are satisfied by the Mendelian population in question.

Definition of Terms

It is necessary at this time to define a few terms which are used in this thesis.

1. Mendelian populations are communities of sexually reproducing and cross-fertilizing organisms integrated by bonds of mating and parentage. These populations are usually, though not necessarily, polymorphic. These populations are also defined as "reproductive communities of individuals which share in a common gene pool", Dobzansky[21].

2. Heterosis...This means the superiority of heterozygotes over homozygotes. (da Chunha[16])

3. Genetic Drift...This is random variation of gene frequency in small populations merely as a consequence of chance. (Ludwin[41])

4. Balanced polymorphism... If two gene alleles, or chromosomal variants, A^1 and A^2, form a heterozygote A^1A^2 which is adaptively superior to both homozygotes, A^1A^1 and A^2A^2, natural selection will tend to establish an equilibrium state, at which A^1A^1, A^1A^2, and A^2A^2 will be present with certain definite frequencies. (Dobzansky[21])

STATEMENT OF THE PROBLEM

STATEMENT OF THE PROBLEM

To find out what happens in a population to mutant genes for ebony body, scarlet eye, and vestigial wings when they are in competition with wild alleles at constant temperature. What changes in the gene frequencies of two alleles occur with variation in temperature and other factors such as food abundance or scarcity, and size of population. To test Hardy's law in a controlled laboratory conditions using Drosophila melanogaster.

APPARATUS AND METHODS

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A. Apparatus:

1. The population cage (see plate 1) used in these experiments was of a simple design and construction. It was an oblong glass cage with a wooden platform. The four walls were made of glass. It could be opened by sliding the end glass wall up through the slit in the wooden frame, and it could be closed by pulling it down. The length of the cage was 25.75 inches, the breadth was 18 inches, and the height, 16 inches. The wooden platform and frames supported the glass top and sides. The platform extended 3 inches beyond the base of the cage, thus making it easy to handle.

2. The cage was kept in a constant temperature room with a temperature of 25.5°C (±1.0°C) during the first fourteen generations of the experiment with ebony. But during the F_{15} to the F_{20} generations inclusive this cage was in a constant temperature room having a temperature between 13.3°C and 18.9°C (±1.0°C). In the first fourteen generations, the room was kept dark at night and semi-dark at day, by drawing the window shade. The constant temperature room in which generations 15, 16, and 17 were raised was dark day and night. But during the 18th to the 20th generations this room was lighted

day and night. All the generations in the experiments with scarlet and vestigial were kept in a constant temperature room with a temperature of 25.5°C (±1°C). The room, in their cases, was dark at night and semi-dark at day.

3. The food on which the flies fed throughout the experiments was of the cornmeal-molasses-agar type. The ration was made according to the following formula:

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

The food was contained in 1/2-pint milk bottles and in petri dishes.

4. The stocks of Drosophila melanogaster came from the General Biological Supply House, Chicago. The genes selected for study were ebony body, scarlet eye, and vestigial wing, which were mentioned in the introduction. In the first generations of all the experiments, 1/2 pint milk bottles, filled to a depth of 28 mm. with the medium, were used to raise the flies before they were put into the cage with petri dishes, on which the F_1's laid eggs which developed into the F_2 animals. The petri dishes were about half filled with the medium.

B. Techniques:

1. The initial crosses between virgin female ebony, or scarlet or vestigial and wild males were made in the half pint milk bottles. The P_1's were removed and discarded when the F_1 pupae appeared. In each case two of these half-pint bottles with the cotton stoppers removed, with F_1 pupae were placed with two petri dishes containing the medium in the cage. When the F_1 flies hatched out in the cage they were allowed to breed at random and to lay eggs on the food in the petri dishes. When the F_2 pupae appeared in the petri dishes, these petri dishes were removed from the cage, the F_1 animals were etherized, killed and discarded.

2. To remove the petri dishes, the cage was placed in a dark room and was then covered with a large paper box. A hole had been made at the top corner of the box. A lighted bulb was placed near this opening in the paper box. In about fifteen minutes the flies had gathered at the top of the cage where the light was strongest, because these insects are positively phototropic and negatively geotropic.[42] Another way to make these animals migrate from the floor to the top part of the cage is to raise one end of the cage about an inch from the table and let it drop with a gentle bang. When the flies had moved up towards the light the cage was opened by raising the end glass just enough to reach in and remove the petri dishes containing the eggs,

larvae, and pupae which gave F_2 generation. Each petri dish was immediately covered with the sterilized top. I estimate that not more than 4% of the flies were lost by this method of removal. After the removal of the dishes, cotton wadding soaked in ether was placed in an extra lid of a petri dish in the cage which was then tightly closed. After the flies were etherized, the cage was opened and they were swept out. The cage was then washed with soap, rinsed with cold water, and left to dry. After drying it was sterilized with 70% alcohol. This concentration of alcohol was used because it is a better sterilizing agent than 95% alcohol. It is not toxic to the animals.

3. The next step was the resetting of the cage. The floor of the cage was covered with sterilized yellow paper. The petri dishes carrying the F_2 pupae were placed inside the cage, then two petri dishes, with fresh food, were placed in the cage with the ones containing the pupae. But these two fresh dishes were placed nearest the end where the cage would be opened, so that they would be removed easily when this procedure was repeated. The fresh dishes were then opened and sterile strips of paper were placed on the medium to provide pupating places for the larvae. The cage was then tightly shut. In about two days the F_2 flies hatched. They were allowed to breed at random, and to deposit their eggs on the fresh medium. When new pupae began to appear in the fresh medium, the process of opening the cage, removing the petri dishes, and etherizing the

animals, was repeated exactly as described above. The etherized F_2 flies were then removed, and classified according to their traits. The cage was cleaned and reset in the manner previously described.

4. The quantity of food was sometimes reduced by supplying only one instead of two or three fresh petri dishes of medium. This was done to see what effect the amount of food had on the frequencies of the phenotypes. The results of generations two through twenty for the ebony X wild crosses are summarized in Table I, fig. I; for scarlet X wild crosses in Table IV fig. 2; and for the vestigial X wild crosses in Table V, fig. 3. The method used enabled the experimenter to keep generations separate. It was used throughout the experiments. Each generation required normally ten days, except in temperature reduction.

OBSERVATION AND DATA ON EBONY

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A. Distribution of Phenotypes in Successive Generations:

1. Tables.

Table I shows the total number of animals raised in each generation, the number of ebony bodied flies and their frequencies obtained experimentally. The expected equilibrium frequency of .25 for the recessive ebony, according to Hardy's Law, was not realized. The ratios were modified even in the F_2, the frequency of which was 23.52%. An equilibrium of short duration seemed to be established for generations F_3 and F_4 with 18.3% and 18.9% of ebonies, respectively. This type of temporary equilibrium was also observed by Shell[55]. The tendency, theoretically, is for the wild and recessive ebony phenotypes to be at equilibrium according to Hardy's Law, but owing to selection, the theoretical equilibrium was disturbed, so there was no permanent equilibrium at any value. After the F_4, the deviations from the expected 25% of ebonies were very significant. The frequency of ebony in F_7 was only about a half that of F_6, that of F_8 about half of F_7, but that of F_9 was about double that of F_8. It could be seen that the experimental ratios grew less and less than the expected 25%. The decline was not consistent. Sometimes the value was greater than the preceding

one and sometimes it was less. Such erratic changes might be due to genetic drift (Wallace[61]) but this might not be the only cause. The changes observed in the experimental populations were in the main such as might be produced by natural selection.

2. Temperature.

The frequency of ebony rose markedly from 5.2% in F_9 to 20.6% in F_{10}. This happened when the temperature regulating mechanism went out of order, so that the temperature went down from 25.5°C (±1 C) to about 15.5°C. This suggested that temperature variation might be a factor modifying the ratios expected under Hardy's Law (Spiess[50], Dobzansky[10], Wallace[61]). Consequently, the experimental cage was placed in a colder constant temperature room with an average temperature of about 16°C, beginning with F_{15}. Before the cage was placed in the colder room, the mechanism which went out of order in the other room was repaired and the former temperature, 25.5 (±1°C), was restored for four generations (F_{11} - F_{14}). As Table I shows, when the temperature went up again to 25.5°C (±1.°C) the frequencies of ebony declined from 20.6% in F_{10} to its lowest point of 0.5% in F_{14}. When the cage was placed in a constant temperature room at about 16°C, the frequency of ebony increased from 0.5% in F_{14} to an average of about 4.0% in F_{15} - F_{20}. Apparently the low temperature influenced the frequency of the ebony phenotypes.

3. Food Factor.

The ration on which an organism feeds has a certain effect on the gene frequency, according to da Cunha[17], who said that the relative adaptive values of ST homozygotes, ST/CH heterozygotes, and CH homozygotes vary depending on the microorganism used in the food of the flies and their larvae. In some populations, he said, the heterozygotes showed heterosis. In others, they were inferior to the ST homozygote. This shows that different gene arrangements make the carrier adapt to different foods. Adaptability of ebony to the food used in this experiment was not studied. The abundance of food may also have an effect on the gene frequency in a population. (Lack[39].) The variation in the gene frequencies of ebony in the F_9 - F_{15} might also have been partially due to quantitative variability of food supply. When a smaller population of flies from F_9 laid eggs on two petri dishes, the frequency rose in F_{10} to 20.6% as already stated. When one petri dish only was used, in F_{11}, for a larger population, the frequency declined considerably in F_{11}. Again when two petri dishes of food were used in F_{12}, the frequency rose by 3.85%. The column labelled "No. of Dishes", Table I, shows the number of petri dishes used in producing that generation. This enabled the author to note the possible effect that food abundance or scarcity had on the size of the populations and on the gene frequencies of ebony, when in com-

petition with "wild type". When one petri dish was used, the amount of food was too small, and the greater competition in the larval stage operated against ebony. (Wallace[61])

4. Duration of Each Generation.

The duration of development of each generation varied from 7 - 19 days. This was influenced by the variations in temperature. It was also influenced, undoubtedly, by the physiology of the internal environment of the experimental animals. (Spiess[51].) The F_3 generation provides an example. The larvae crawled over the floor and the walls of the cage and the development took a longer time than expected.

5. Fecundity or Productivity of Females.

The result of the experiments which were conducted to test the fecundity of the females are given in Table II, Parts I - III. From the results of these experiments, there is an indication that the numbers of flies raised for 20 days from a pair of females of each phenotype, vary. The wild produced more offspring than ebony. Table II, Part I. Apparently these results are not a true measure of the productability of the phenotypes. Genetic consequences of fecundity do not depend upon egg production per female alone. Wallace[61]. Other factors, such as number of females, which is governed by longevity, are also involved. Most of the eggs laid also perished, as pointed out before. Habitability? of the eggs is a factor which determines the number of organisms which may enter the larval phase.

Competition in the larval stage, and finally the successful emergence of the imagos from the cocoon all determine what the number of individuals realized from a female or a pair of females would be. The data obtained in these fecundity experiments are very scanty, and therefore do not tell much about these animals' fecundity. It would appear, however, that fecundity of wild was greater than that of the ebonies (Table II, Part II), but I do not know why. All the fecundity experiments were conducted at a temperature of 25.5°C (±1°C).

6. Sexual Activity Experiments.

According to Wallace (61), in a population of sexually reproducing individuals the transmission of chromosomes and genes from one generation to the next depends, in part, upon the activity that various individuals show in the acquisition of mates. Genes which decrease or increase the sexual activity and capability of their bearers correspondingly alter their frequency of transmission. Table III, Parts I and II, gives the results of assortative or selective mating experiments which were made in this research. Merrell[43] has clarified the terms "assortative" and "selective mating". Quoting other authors he said that "positive assortative mating", or "courtship discrimination" leads toward sexual isolation, while "preferential mating" differences in "sexual drive", "differential mating propensities", "one-sided mating preferences",

or "selective mating" produce changes in the relative frequencies of the genes involved, and hence must be considered as a phase of natural selection. In Table III, Part I, the ratio of the wild animals to that of ebony is 1:2. It means, all things being equal, the ebony female mated twice as much with the ebony male as she mated with the wild animal. In Part II of this table, when there were two females (one ebony, the other wild) with one ebony male, the ratio of the wild flies to ebony was 1: 1.3. This means the ebony female mated 1.3 times as much with the ebony male as did the wild female. The ratios in both parts are not exactly the same, probably because in Part II experiment, the liberty of the ebony female to mate with the ebony male was interfered with by the presence of the wild female. The results of the experiment do not show clearly whether the male or the female determined the occurrence of mating. Nevertheless the results seem to indicate the selective or preferential mating.

B. Other Experiments:

Originally, populations of wild X ebony flies were to be studied to see whether the theoretical Hardy equilibrium would be established at the expected ratios. But it was observed that even in the F_2 and the generations following, the expected ratios were different from those expected from Hardy's Law. The experimenter thought and was advised

by Dr. Hunt to devise experiments to determine why this was true. And so the experiments on fecundity and sexual activity, already mentioned above, were conducted in 1/2-pint milk bottles with the same type of food as was used in all other experiments in this research.

In the first fecundity experiment, two virgin ebony females were placed with two ebony males in a 1/2-pint milk bottle and were allowed to mate normally for six days. When pupae appeared in this bottle, the P_1 ebonies were removed and discarded. The pupae hatched into F_1's and counts were kept of the number of animals produced in 20 days from this mating. The data for this experiment are summed up in Table II, Part I.

Similar crosses were made with two wild virgin females, and two wild males to determine the fecundity of homozygous wild in comparison with ebony mutants (see Table II, Part II, mentioned before).

Crosses between heterozygotes were also made in exactly the same way as was used in ebony X ebony and wild X wild crosses. The results for these heterozygotes are tabulated in Table II, Part III.

1. Selective mating.

Experiments were also conducted to find out whether there was selective or assortative mating. In Part I of this experiment, one virgin ebony female, about 48 hours old, was put in a half-pint bottle with two males, one

ebony and one wild. When pupae appeared in this bottle the three animals were removed and discarded after six days, and the pupae allowed to hatch. Counts were taken for 12 days. The results are shown in Table III, Part I. Part II of this shows results of the reverse crosses. In this case instead of having one wild male and one ebony male with one virgin ebony female, two females (one ebony, one wild) were placed with one ebony male in a half-pint culture bottle, and allowed to breed. The same procedure of Part I was used here, and the insects were allowed to breed for 12 days.

In trying to find out what factors were modifying the frequencies of mutant sepia eye, Sara Shell[55] had made experiments similar to the ones used above.

2. Temperature.

As said before, the regular constant temperature room, where most of this work was done, had a temperature of 25.5°C (±1°C). But for a whole week the mechanism for keeping this temperature constant went out of order, in the winter, and the room was very cold, the temperature then being about 16°C. The writer found that during this period of accidental cold, the frequency of mutant ebony, which had gone down to 5.2% in F_9, went up to 20.6% in F_{10} (Table I). It was thought that probably temperature was a factor which modified the expected ratios according to Hardy's Law, and so a constant temperature room mentioned above,

was secured in which the animals were raised in a temperature of between 13.3°C and 18.9°C instead of 25.5°C (±1°C). Table I, generations 17-20, shows the effect of varying the temperatures.

C. Graphs:

Figures 1, 2, and 3 show the expected curves according to Hardy's Law and the experimentally determined curves. The expected and the experimental curves do not coincide because of selection pressures in the experiment. Figure 1 corresponds to Table I, figure 2 to Table IV, and figure 3 to Table V. In all the graphs, generations on the X - axis, are plotted against phenotypic frequencies on the Y - axis.

DISCUSSION ON EBONY.

DISCUSSION ON EBONY

The main purpose of this experiment was to test Hardy's theoretical equilibrium law in laboratory with Drosophila Melanogaster populations. An average of a little over 1000 flies ~~was~~ raised per generation. These animals were classified phenotypically into ebony body and wild. Some collateral experiments were devised to test the effect of temperature, selective mating, and differential fertility on the phenotypic frequencies of the recessive ebony trait. Experimentation with quantity of food used was to determine its effect on the gene frequencies. Even as the experiment was beginning with the F_2 generation, it was seen that the distribution was not according to Hardy's Law. This was an evidence that the ratios were modified by certain factors. The result was not an invalidation of the law. It was an indication of the action of natural selection against the equilibrium state. Natural selection is a vehicle of organic evolution. In a natural environment selection is going on all the time. The least fit are selected against. They pass out of existence, and the fit - test perpetuate the race. Heterozygotic superiority of any species is a preserver of a certain proportion of both the dominant and recessive traits in the species. Balanced polymorphism thus maintains an equilibrium state in a community of random breeding organisms in nature. In the lab-

oratory the behavior of the animals can be duplicated. In this duplication, factors which in the laboratory, as in nature, disturb the equilibrium are revealed. These factors will now be discussed.

1. Temperature as a factor.

Dobzansky[9] and other investigators cited already have shown the effect of temperature on the frequencies of chromosome inversions in Drosophila. Spiess[50] found that there was a difference in gene frequencies of chromosomes WT and WD when the flies containing these chromosomes were raised at temperatures of 25°C and 16°C. At a temperature of 25°C the frequencies of these chromosomes from generation to generation were constant, but at 16°C there was a significant change in the frequencies of these chromosomes, WT increased nearly 20% with a drop in WD over a period of three generations. He found that a new equilibrium level was reached at 58% WT, and 42% WD.

Anderson[2] found that a certain gene concerned with the fertility of Drosophila Melanogaster had its major effect on its normal allele at a low temperature of 19° ± 1°C. He found that a certain genotype lz^{36}/ lz) was very sensitive to temperature changes, with the sensitive period occurring at about the time of pupation. He summarized that the infertility of lz^{g} females (and probably other lozenges) was apparently due to a number of factors including a short copulatory time, smaller initial sperm storage, and degeneration of sperm within tubular receptacle of the females.

Wallace[61] has shown, in his experiment with "Sex Ratio" in Drosophila Pseudoobscura, that in competition with the

ST chromosome, the SR chromosome decreased in frequency. He found that whereas SR was eliminated at 25°C it was retained at $16^{1/2}$°C. When he analyzed the physiological properties of the flies, he discovered that at 25°C the adaptive value of SR/SR (homozygous) females was close to zero, hence their contribution to the gametic pool of the population was extremely limited; SR males contributed less than ST males. The superiority of the SR/ST (heterozygote) females at 25°C tended to establish an equilibrium, but the inferiority of the homozygous females and of SR males was sufficient to upset this tendency and to result in the elimination of SR males from the population. At 16 $^{1/2}$°C, he said, the contribution of SR males to the gametic pool was higher than that of ST males. The adaptive value of SR/SR females, though low, was substantially greater than zero. Consequently, he said, SR was retained in the populations at this temperature (16 $^{1/2}$°C).

As already shown in this research, it was found that when the temperature of the room declined to about 16°C, the frequency of recessive ebony increased from 5.2% in F_9 to 20.6% in F_{10}. This significant rise was not a matter of chance, but was due to temperature change. When the temperature was restored to about 25.5°C the frequency of ebony went down again, but this time to 10.55% in F_{11}. It went down 51% approximately. At 25.5°C the downward trend of the gene frequency of ebony continued to its

lowest level in F_{14} with a class frequency of 0.5. But when the cage was placed in a constant temperature room with temperature of about 16°C the frequency of ebony rose again to a new level in F_{15} with a frequency of 3.4%. This new level was approximately maintained at 16°C until the 20th generation (Table I). Temperature was, therefore, a factor which disturbed Hardy's equilibrium, with respect to the ebony gene.

2. Differential Productivity or Fertility as a Factor.

Anderson[2] found that sperm mobility and genitalia abnormalities were functions in the fertility of the lozenge female in D. melanogaster. He said that the abnormalities of spermathecae and parovaria in lozenge females suggested that their low fertility might be related to sperm content of the females. He said that Oliver and Green found that homozygous lz^g females produced many eggs over an extended period of time, but the fertile eggs are few in number and appeared only during the first few days of copulation. They suggested that the phenomenon might be due to possible loss of sperm viability in the ventral receptacles of the females.

Wallace[61] said that in a continuous population such as was used in this research, the females influence the chromosomal or gene frequencies through the eggs they lay. The greater the number of eggs they lay, the greater their contribution to the succeeding generations, if everything is equal. Selection acting upon the frequencies of reces-

sive ebony gene or its "wild type" allele in the experimental population, then might have done so through differential fecundities, fertilities, or productivities of the three types of females, the dominant and recessive homozygous and heterozygous females in each generation.

To learn the relative productivity of ebony and "wild type", three experiments were set up. They were to show the productivity of homozygous recessive ebony, homozygous dominant wild, and of the heterozygotes. The results of these experiments are shown in Table II, Parts I, II, III. Judging from the number of offspring produced in 20 days, from a cross of 2 males and 2 females in each case, there is a difference in the apparent productivity of these phenotypes. In this period 634 ebonies, 705 wilds, and 684 flies from the heterozygotes were produced from the crosses of 2 ebony ♀♀ X 2 ebony ♂♂, 2 wild ♀♀ X2 wild ♂♂, and 2 heterozygote ♀♀ X 2 heterozygote ♂♂, respectively. This is a ratio of about 1: 1.1: 1.06. The number of offspring raised might have been determined by differential mortalities in the egg, larval, and pupal stages. The next factor then will be.

3. Differential Mortality.

Da Cunha[18], experimenting with D. Polymorpha, found that although eggs were deposited abundantly, many larvae left the food, wandered on the glass of the culture vessel, and died before pupation. The mortality of the genus Drosophila may occur at any stage; egg, larva, pupa, or adult.

This same investigator said, in attempting to clarify the mechanism of action of the selective process, the simplest hypothesis would be that the differences in the adaptive values of the three genotypes (Dominant homozygote, heterozygote, and recessive homozygote) result in differential mortality between the egg stage and the adult. He also found that differential mortality between the egg and the adult stages favored heterozygotes in the laboratory culture bottles, and also, under same conditions, in experimental populations. But in natural populations, which evidently exist in environments different from the laboratory cultures, differential mortality favors homozygotes. The agency which is responsible for this difference is not known. Nutritional difference has been suggested as a possible agency.

Although egg, larva, and pupa counts were not kept in all generations of the present experiment, it is, however, safe to say that from a rough estimate, only about 45% of the eggs laid reached the adult stage. By inspection it would seem that not all the larvae pupated. In F_3, Table I, it was found that a great many of the larvae crawled out of the culture dishes and pupated on the floor and walls of the cage. Such pupae seldom succeeded in emerging from the cocoon. This happened, not only in F_3 but generally, though it was more noticeable in F_3. It is therefore reasonable to suggest that the modification of the expected gene fre-

quencies might have been due in part to mortality at different stages of development of the flies.

4. Selective Mating.

Sexual activities of any sexually reproducing organism are one of the most important factors affecting gene frequencies. The sexual activity frequently encountered in populations of Drosophila is assortative mating which was referred to before under "Sexual Activity". This type of mating behavior is said to lead toward sexual isolation. Wallace[61].

Another type of mating behavior which is important is "preferential mating" which is also called differences in "sex drive", "differential mating propensities", "one-sided mating preferences", or selective mating. Wallace[61] says preferential or selective mating produces changes in the relative gene frequencies of the genes involved and hence must be considered as a phase of natural selection. As already pointed out under "Observation and Data on Ebony", it was found experimentally that ebonies preferred ebonies, and this might have been a very important factor which modified the ratios expected from Hardy's Law.

Rendel[49] said that in most cases it is the female that refuses to take part in the courtship, the male being prepared to court with almost anything. This is not always the case, because it has been found that Drosophila affinis and Drosophila algonquin courted females of the same species more readily

and more persistently than those of the other. Rendel[49] showed that in Drosophila subobscura yellow males preferred yellow females, though yellow females mated normally. Rendel also said that mating of homozygous males depends on whether mating is carried out in the light or dark. He said illumination affected their fitness. He found that the proportion of female vestigials inseminated in the light was 49.4%, and in the dark 46.6%. Ebony inseminated 21.5% of females in the light and 60.7% in the dark. His experiment showed that ebony was much more successful in mating in the dark than in the light. Rendel found also that competition was between two types of females for the same type of male, not between two types of males. In the experiments on which this thesis is written, Table III, Part I shows that competition was between ebony male and "wild-type" male for one type of female, ebony female. The results show that either the ebony male had the upper hand or the ebony female preferred ebony males. In Part II of the same table the competition was between two females, wild and ebony. Again, according to the results in Part II of this table the mating frequencies of ~~wild~~ ebony female with ebony male were higher than mating frequencies of wild female with ebony male. In view of the fact that these matings were made in the dark, in the cabin, ebony was more successful because, according to Rendel, already cited, ebony is more successful in the dark than in the light, and wild more successful in the light than in the dark. This does not preclude the fact that selective or preferential mating was also at work, Rendel[49]. It is to be pointed out, however,

that the number of matings used in preferential mating experiments was too small to justify conclusions. The results obtained in these experiments are suggestive, not conclusive.

CONCLUSION ON EBONY.

CONCLUSION ON EBONY

1. According to the data obtained from the series of experiments conducted with ebony X wild crosses, it was found that the Hardy equilibrium was not established as expected.

2. In competition with "wild type", the ebony gene declined progressively, but it was not entirely eliminated from the populations.

3. The failure to get Hardy equilibrium was due to selection pressures.

4. Temperature was found to be one of the factors which disturbed the expected equilibrium from Hardy's Law. At a temperature of about 16°C recessive homozygous ebony survived better, and its gene frequency was higher than at 25.5 C ($\pm$1°C).

5. Another factor which appeared to modify the equilibrium ratios was selective mating. It was found that ebonies preferred to mate more with ebonies than with "wild type".

6. Differential fertility or fecundity might be suggested to have played a part in the modification of the expected ratios.

7. The data obtained on selective mating and fecundity are so scanty that the results are suggestive rather than conclusive.

8. The variations in the phenotypic frequencies of ebony from generation to generation seemed to indicate genetic drift.

OBSERVATION AND DATA ON SCARLET.

OBSERVATION AND DATA ON SCARLET

A. Table IV and Figure 2:

Table IV gives the results of the experiment with the scarlet eye. Figure 2 is a graphic representation of the data shown in this table. Twenty-six generations of flies were raised. These generations on the X axis are plotted against the class frequencies of recessive scarlet on the y-axis.

In the 4th generation, some of the flies mutated to white eyes, a trait which persisted until the 14th generation. The percentage frequencies of mutant white eye are also plotted on the y axis against generations on the X axis in figure 2. The class frequencies of all the mutant phenotypes dealt with in this research were expressed in percentages.

B. Comments on Table IV and Figure 2:

An average of about 1000 flies was raised per generation. This large number was essential in order to meet the condition specified in Hardy's Law[29]. The procedure by which the data in this table and graph were obtained was exactly the same as used in the ebony X wild and vestigial X wild experiments. The same type of cage (see the plate), food, bottles, and petri dishes were used. The constant temperature room maintained a temperature of 25.5°C ($\pm$1°C) all through the 26 generations. At the

beginning three petri dishes of fresh food were supplied to each generation of animals, but later one or two petri dishes were used to rate the effect of quantity of food on the population and on the frequency of recessive scarlet eye. Each generation lasted about ten days.

C. Food Factor:

Da Cunha[16] has shown, as said above under ebony, that the genes carried on the chromosome makes the carrier adapt to different foods. Abundance or scarcity of food is a food factor which may affect the gene frequency of a population. Lack[39]. In this experiment food abundance as revealed in Table IV did not make much difference in the number of flies in the population in each generation. Whereas in the F_2 1600 animals were raised from 3 petri dishes, in F_{12}, 2000 were raised from 2 petri dishes, and in the 14th generation 2270 from one petri dish. The gene frequency of scarlet seemed to decline progressively first without a cut in the quantity of food, then with reduction in the amount of food used.

D. Differential Equilibria:

According to the data in Table IV, equilibria seemed to have been established at different levels. There was a kind of equilibrium from the 3rd to the 6th generations, from F_{16} to F_{21}, and from F_{22} to F_{24}. Probably these levels were pseudo-equilibria.

E. White-eye Mutation:

Mutation to white eye was observed in the 4th generation, but was eliminated in the 14th. Mutation is said to have some effect on the gene frequency of a Mendelian population such as this. Wallace[61] says that mutation pressure is one of the agencies which may alter the frequencies of chromosomal types in populations.

The elimination of the white eye was to be expected. The Reeds[47] demonstrated in the laboratory that the white eye in competition with the "wild type" was completely eliminated.

DISCUSSION ON SCARLET.

DISCUSSION ON SCARLET

The results obtained show that, in competition with "wild type" allele, the gene for scarlet eye decreased in frequency at a temperature of 25.5°C (±1°C). Since there was no temperature variation, the effect that temperature might have had on the frequency of scarlet cannot be determined here.

Equilibrium

Was equilibrium established according to Hardy's Law? No. Deviations were noticed in all generations due to selection factors. But this does not mean that Hardy's theory is invalid. It means that the ideal conditions which are assumed by Hardy were disturbed by some agencies. There are several agents or factors which may account for altered chromosomal and gene frequencies in Mendelian populations. These agents are mutation pressure, genetic drift, selection, and migration (if in a natural environment). Environmental factors such as temperature variation, (Spiess[50], Dobzansky[9]) humidity, population density, quality and quantity of food, food availability and scarcity, sexual activities of the organisms concerned (Wallace[61]) fecundity of the females, proportion of the females in the population, larval competition, longevity of the adults, hatchability of the eggs, and differential mortality have been known to be the means through which natural selection

brings about the changes in the chromosomal and gene frequencies in Mendelian populations. In such populations natural tends to establish an equilibrium state at which the genes for dominance and recessiveness will be present with definite frequencies. This state of equilibrium is known as balanced polymorphism. Dobzansky.[21] The word "tend" is used advisedly. Natural selection will "tend" to establish an equilibrium which variable factors work to de-equilibrate.

Reference to Table V shows us what we mean by "tend" here. We at once see that natural selection tended to establish an equilibrium between "wild type" and scarlet genes at different intervals as brought out before. Between F_4 and F_6, F_{16} and F_{21}, F_{22} and F_{24}, this tendency to establish equilibrium is possibly evident.

Food and Population Density:

Quantity of food, (Lack[39],) and type of food, (da Cunha[16],) may affect the gene frequencies of organisms. The type of food used in this research was of cornmeal - molasses - agar, the formula given under "Apparatus". The quantity of food did not seem to have had much effect on the size of the population, but perhaps it affected the gene frequency as a consequence of competition. In F_7 when the population dropped by almost a third from 1150 to 785, the phenotypic frequency went up from 17.57% to 27.74%. But in F_8 when the population declined further to the lowest level (195) the phenotypic frequency was 23.59%. In all these generations the quantity of food was the same. As the population in-

creased again from F_9 through F_{26}, phenotypic frequency declined progressively until the lowest frequency in F_{26} when the experiment was terminated at the end of the school year. It may be suggested that the decline in the frequency was a function of both the population density and the reduction in the quantity of food to one petri dish.

CONCLUSION ON SCARLET.

CONCLUSION ON SCARLET

A. It has been shown from the data obtained from this experiment that in competition with the "wild type", the recessive scarlet gene declined progressively.

B. The expected equilibrium according to Hardy's Theory was not established due to modification by selection pressures. But in several generations temporary equilibria were found.

OBSERVATION AND DATA ON VESTIGIAL.

OBSERVATION AND DATA ON VESTIGIAL

A. Table V and Figure 3:

The data in Table V give information about the vestigial X wild random mating experiment which was carried on for 12 generations. Figure 3 represents these data graphically. The phenotypic frequencies of the vestigial are given on the y - axis and the generations on the abscessae. Over one thousand animals were raised in each generation.

The vestigial phenotypes disappeared entirely in F_4 - F_6 generations but reappeared in the F_7 and continued till the termination of the experiment.

A glance at the data tells one that vestigial was severely selected against even in the second generation. In this second generation the ratio of the frequency of the "wild type" to vestigial should be 3:1, because vestigial is simple Mendelian recessive. Instead the ratio of the vestigial was only half of the expected (12.6%). It was clear at once that in competition with the wild, vestigial was drastically at a disadvantage.

B. Mutations Observed:

1. In the second generation (Table VI) mutations to white eye and ebony-like body were observed, but in the third generation the white eye trait was eliminated as was the white eye mutation in scarlet X wild experiment discussed.

In the 4th generation the ebony-like body mutation was also eliminated. The cause of the disappearance of ebony body-like trait is unknown.

2. Mutation to Miniature-like Flies

Normally the vestigials have only vestiges of wings. But in the course of this experiment some flies mutated to miniature-like flies. Their wings were much bigger than the vestiges of the vestigial but a little smaller than those of the miniature. This mutation was observed in the 7th generation and continued with decreasing frequencies till the termination of the experiment. (See Table V).

DISCUSSION ON VESTIGIAL.

DISCUSSION ON VESTIGIAL

Factors Responsible For the Decline in Vestigial Gene Frequencies:

1. Viability

The vestigial trait, as mentioned elsewhere already, is a great physical handicap to the organism. The Reeds[47] stressed the importance of physical fitness as a mechanism through which genes exercise their damage. The viability of the vestigial was very low.

It was observed that when the F_2 were emerging from the pint bottles, the vestigials were unable to move out quickly, while the wild ones were flying all over the cage, mating many times with their type.

2. Preferential Mating

Rendel[49] found that both ebony and vestigial males mated preferentially with the females of the type not their own. Although the same author found that vestigial males were more successful in competition with ebony when both were young, it cannot be said that vestigial was more successful than the wild in this experiment. It was less successful due to its physical handicap. Rendel also found that the vestigial was more successful in the light than in the dark. Perhaps during the night when the cage that was used in this experiment was in the dark, the vestigial

could not mate at all. Most of the day the experiment room was also dark, because the blinds were always kept drawn. This, coupled with lack of agility by the vestigial, possibly made selection strongly against it.

3. Temperature

The culture conditions were closely controlled with respect to temperature which was constant, at 25.5°C (±1°C). Its effects on variability of gene frequencies of various arrangements of chromosomes have been demonstrated by Dobzansky[9] and other investigators.

4. Food

It has been brought out that quantitative variations (Lack[39]) and qualitative varieties (da Cunha[16]) of food play important role in the gene frequencies of organisms. The quantity of food was constant in this experiment. The formula of the culture medium used is an index of the quality of food on which these organisms were raised. Da Cunha, Dobzansky, and Sokoloff experimented with food preferences of sympatric species of Drosophila[17]. They found that some species preferred certain kind of foods, and that they differ in their preferences, but that their differentiation did not amount to rigid specialization. These workers therefore concluded that natural selection tends to lessen the competition among sympatric species by making them prefer different foods normally found in their environment.

5. Mutation

It was brought out before, in the scarlet experiment, that one of the agencies which may alter the chromosomal and gene frequencies in a population is mutation. Several mutations were reported in this experiment. But as to whether they affected the phenotypic frequencies of vestigial, I do not know.

CONCLUSION ON VESTIGIAL.

CONCLUSION ON VESTIGIAL

A. Natural selection rapidly eliminated the gene for vestigial wing from the population where there was a competition between "wild type" and vestigial. The reappearance of vestigial in F_7 (Table VI) was probably due to the presence of heterozygotic individuals in the population. The gene for vestigial was not completely eliminated. L'Heritier and Tessier[36] found that the gene for "bar" could not be completely eliminated. These same workers, helped by Neefs[37], found that the gene for vestigial, although severely selected against, was not completely eliminated.

Low viability was perhaps the reason why selection was so much against vestigial.

B. Mutations were observed but I do not know whether they affected the expected ratios according to Hardy's Law.

SUMMARY.

SUMMARY

1. In this research work which lasted over a year three traits of Drosophila Melanogaster were used to test Hardy's equilibrium theory in the laboratory. These traits were ebony body, scarlet eye, and vestigial wing. The genes for these traits are all autosomal. At least an average of a thousand flies were raised in each generation and in each case.

2. One of the facts found common to all three traits is that, in competition with the wild allele, each one of them deviated from the Hardy's expected ratios. The decline of vestigial was the most rapid, due probably to its physical handicap which had been brought out before.

3. The Hardy ratios were not realized because natural selection, working through many agents, selected against the recessive traits in competition with their "wild type" allele.

4. In the case of Ebony it has been found that the following were the agents of natural selection:

a. Temperature, Dobzansky, da Cunha

b. Selective mating

c. Differential fecundity or productivity.

5. The random and erratic variation in the phenotypic frequencies of ebony suggests genetic drift.

6. Scarlet:

a. In the experiment with scarlet, mutation to white eye was found, but as to whether this affected the expected ratios, I do not know.

b. Quantitative variation of food made no difference in the size of the population but perhaps it affected the gene frequency as a consequence of competition.

c. Pseudo-equilibria were observed between F_3 and F_6; F_{16} and F_{21}; and F_{22} and F_{24}.

Vestigial:

a. Several mutations were reported.

b. Viability was probably a factor which affected the equilibrium ratios.

c. Preferential mating was reported on vestigial by Rendel[49]. The author, however, did not experiment with preferential mating in the case of vestigial.

TABLE I

FREQUENCIES IN PERCENTAGES, OF EBONY FLIES AND TOTAL NUMBERS OF FLIES RAISED IN EACH GENERATION

Generation	Total	No.	Ebony Phenotype frequency in %'s	No. of dishes of food	Duration	Temperature 25.5°C (±1°C)
2	1650	378	23.52	2	7 days	
3	1859	353	18.98	2	15 "	
4	1156	213	18.42	2	9 "	
5	1751	202	11.42	2	9 "	
6	1943	189	9.70	2	10 "	
7	2098	95	4.5	2	9 "	
8	1937	54	2.8	2	8 "	
9	516	27	5.2	1	10 "	
10	719	148	20.6	2	8 "	16° C
11	1602	169	10.55	1	10 "	25.5°C (1°C)
12	1650	165	14.4	2	7 "	"
13	1220	58	4.8	1	9 "	"
14	1369	7	.5	2	9 "	"
15	595	19	3.4	1	14 "	16°C (average)
16	1144	59	5.1	2	17 "	"
17	1483	25	1.7	2	9 "	
18	968	62	6.2	2	19 "	
19	734	32	4.4	1	16 "	"
20	662	20	3.0	2	11 "	"

TABLE II

PART I: PRODUCTIVITY OF DIFFERENT PHENOTYPES

CROSSES		Duration	No. of Flies Raised
Females	Males	30 days	634
2 Ebony X	2 Ebony		
PART II.			
2 Wild X	2 Wild	20 days	705
Ratio: 634 Ebony: 705 Wild = 1: 1.1			
PART III.			

Females	Males		Total	Ebony
2 Hetero-zygotes X	2 Hetero-zygotes	20 days	684	128

TABLE III

PART I: SELECTING MATING DATA

Cross: 1 ebony female X male wild and 1 male ebony, for 12 days.

Counts	Wild	Ebony
1	64	135
2	31	43
3	3	8
Total.....	98	186
Ratio.....	1 :	2.

PART II: SELECTIVE MATING DATA. REVERSE CROSS TO THAT GIVEN IN PART I.

Cross: 1 wild female and 1 ebony female X 1 ebony male for 12 days.

Counts	Wild	Ebony
1	59	82
2	19	15
3	26	41
Total.....	104	138
Ratio.....	1 :	1.3

TABLE IV

CLASS FREQUENCIES OF SCARLET EYE FLIES AND TOTAL NO. OF FLIES RAISED IN EACH GENERATION AT TEMPERATURE OF 25.5°C (±1°C)

	Scarlet			Gene Freq.'s	Mutant White		
Generation	Total No. of Flies in Each Generation	Total No. of Scarlet in Each Generation	Pct. Scarlet in Each Generation		No. in Each Generation	Pct. of White Eye in Each Generation	No. of Dishes Used
1			25	.50			
2	1600	327	20.44	.45	-	-	3
3	1811	343	18.96	.43	-	-	3
4	1100	178	16.18	.40	11	1	3
5	2139	344	16.07	.40	60	2.8	3
6	1150	222	17.57	.42	16	1.3	3
7	785	210	27.74	.53	17	2.3	3
8	195	46	23.59	.49	-	-	3
9	824	168	20.38	.45	25	3.0	
10	939	136	14.49	.38	32	3.0	3
11	1820	251	13.8		66	3.6	3
12	2000	230	11.5		-	-	2
13	1550	115	7.4		7	0.45	3
14	2270	260	11.45		14	0.60	1
15	785	126	16.05		-	-	1
16	747	62	8.3		-	-	1
17	1022	84	8.0		-	-	2
18	1713	109	6.3		-	-	2
19	1478	120	8.1		-	-	1
20	1375	107	7.77		-	-	1
21	1036	108	7.00		-	-	1
22	709	23	3.3		-	-	1
23	1826	58	3.1		-	-	-
24	974	30	3.0		-	-	-
25	1317	72	5.4		-	-	-
26	1393	26	1.9		-	-	-

TABLE V

CLASS FREQUENCIES OF VESTIGIAL FLIES AND TOTAL NO. OF FLIES RAISED IN EACH GENERATION

Generation	Total No. of Flies In Each Generation	Vestigial		Mutations Observed	
		Total No. in Each Generation	Percent in Each Generation	White Eye	Ebony Body
2	1853	225	12.6	7	47
3	1105	70	6.3	-	6
4	1123	-	-	-	-
5	1466	-	-	-	-
6	1340	-	-	-	-
7	1250	1	.08	-	-
8	1058	2	.19	-	-
9	1863	10	.50	-	-
10	3082	6	.19	-	-
11	1646	11	.66	-	-
12	1091	2	.18	-	-

FIGURE 1

EXPERIMENTALLY OBTAINED FREQUENCY OF EBONY COMPARED WITH THEORETICALLY EXPECTED

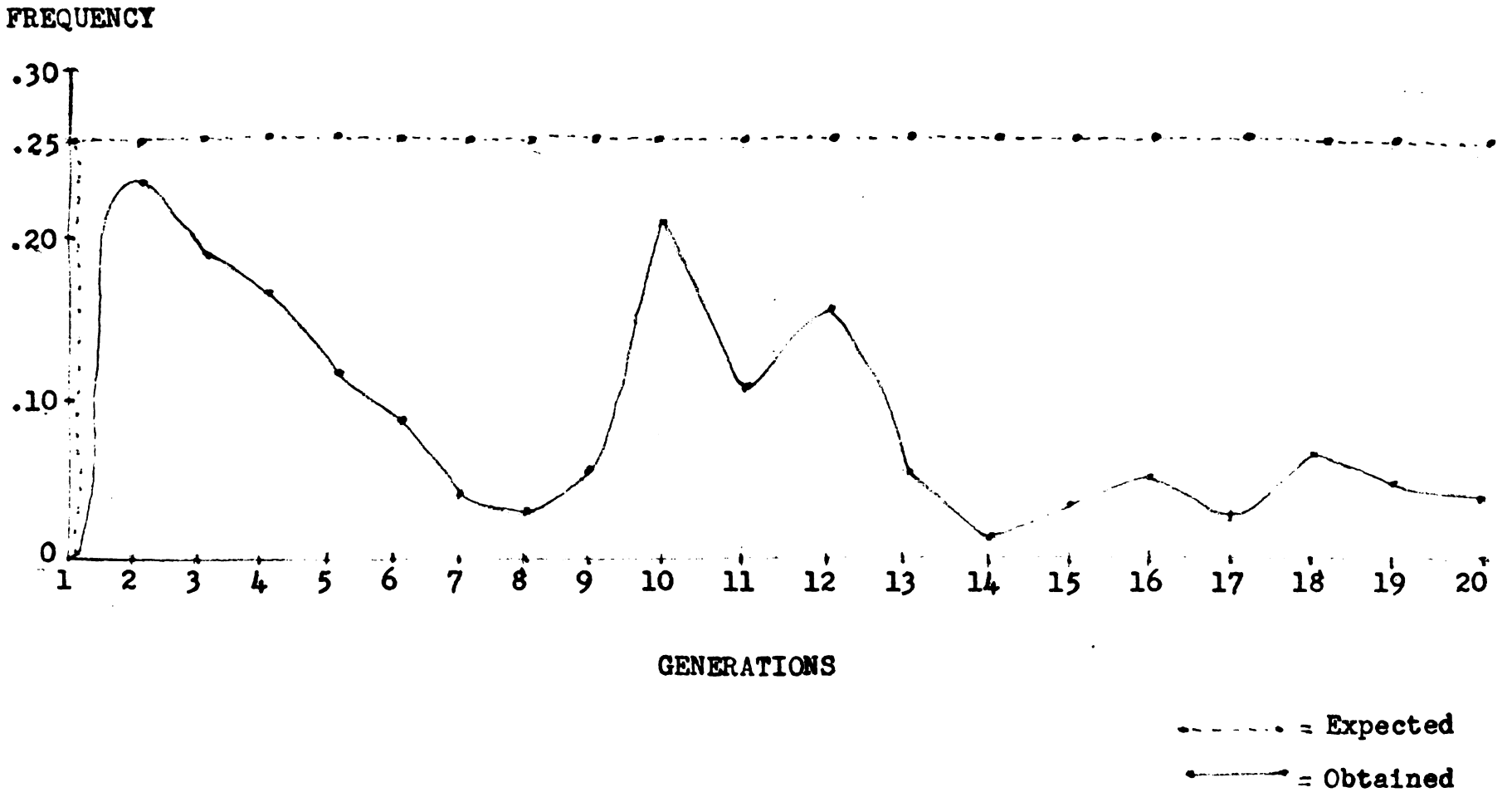

FIGURE 2

EXPERIMENTALLY OBTAINED FREQUENCY OF SCARLET COMPARED WITH THEORETICALLY EXPECTED

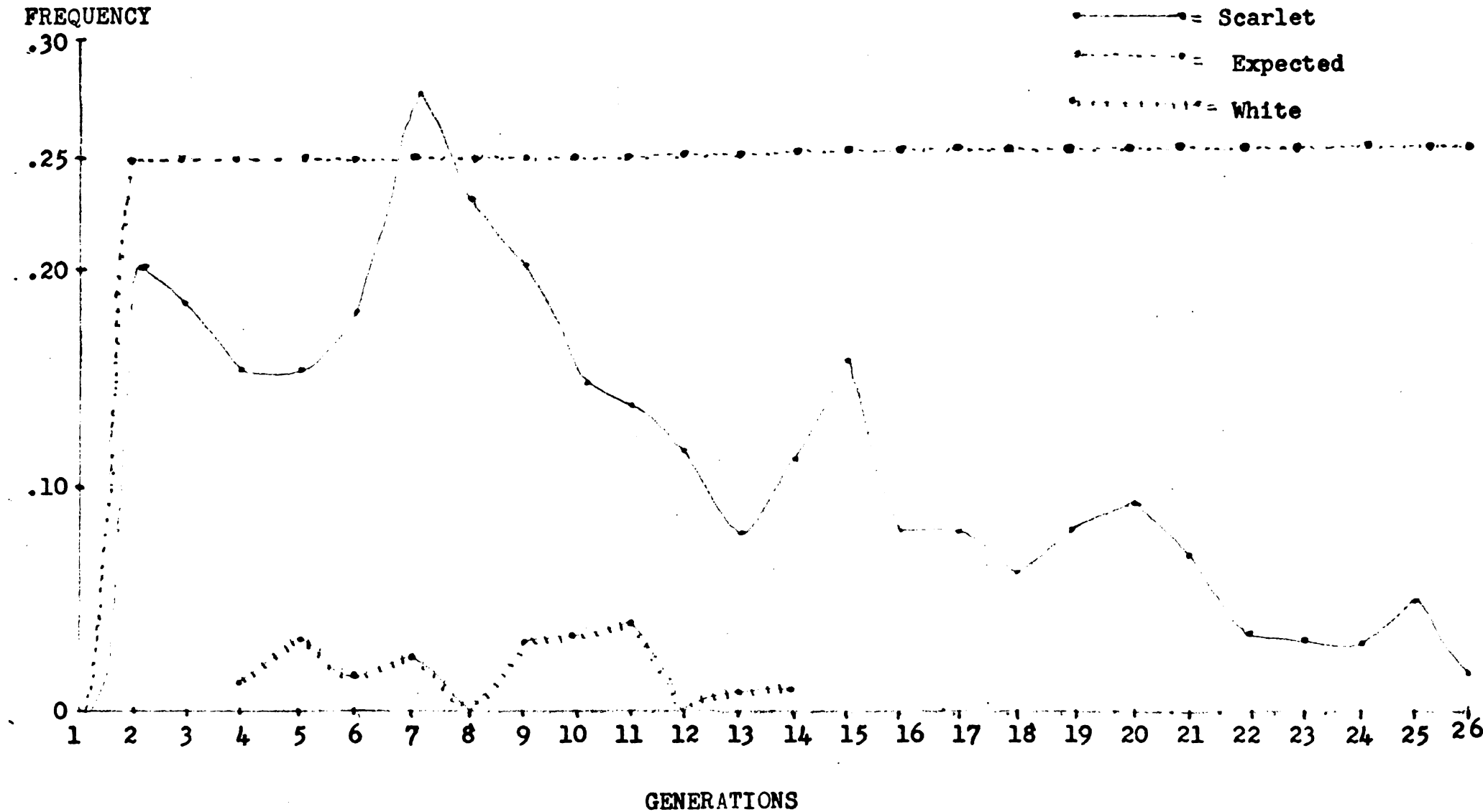

FIGURE 3

EXPERIMENTALLY OBTAINED FREQUENCY OF VESTIGIAL

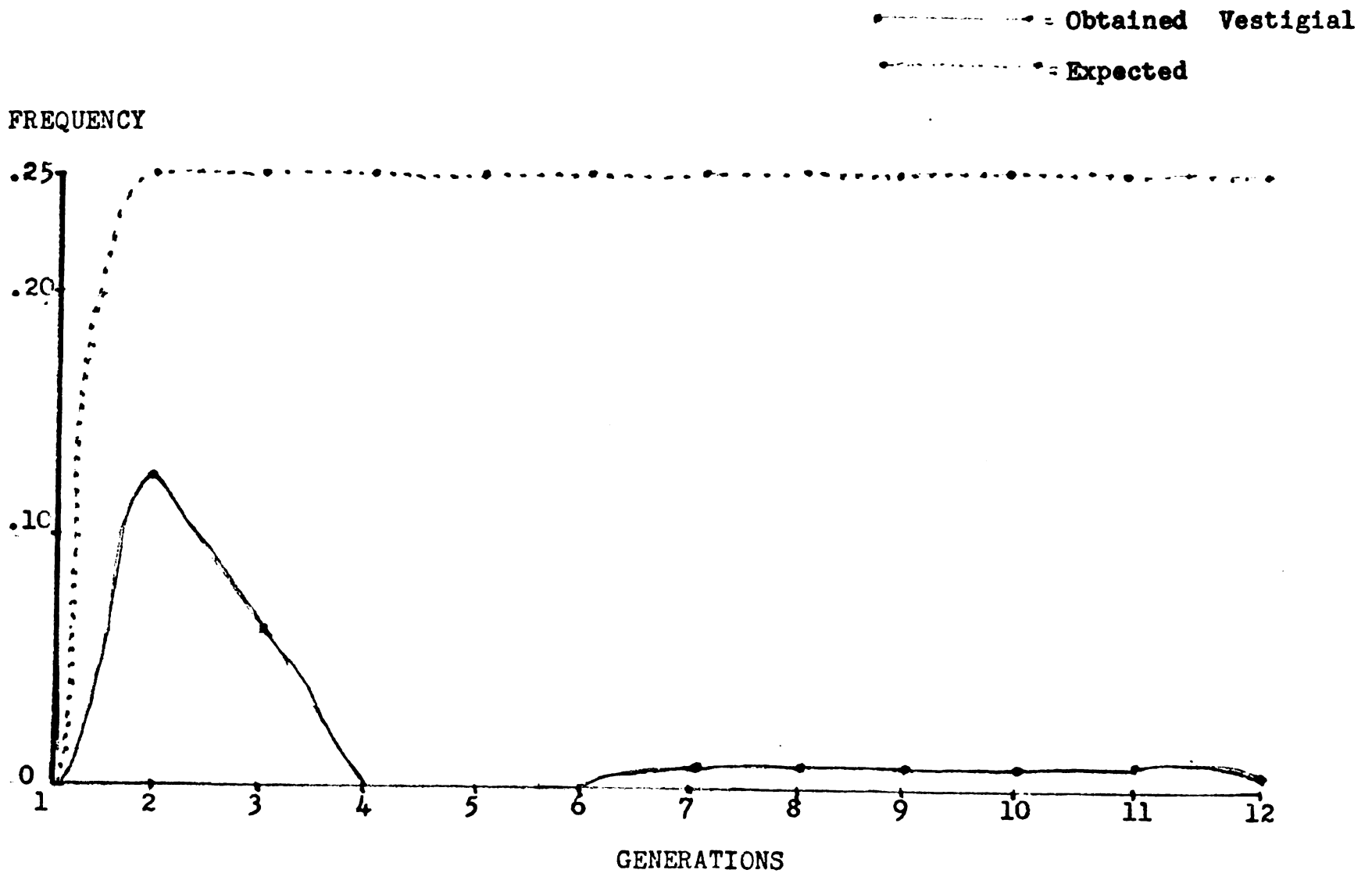

PLATE I. Experimental Cage Used in the Research of this Thesis

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BIBLIOGRAPHY

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