PHENOTYPIC FREQUENCIES IN LARGE, RANDOM-BREEDING POPULATIONS OF DROSOPHILA MELANOGASTER WHICH CARRY THE GENES FOR WILD AND SEPIA EYE COLOR

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By

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INTRODUCTION

INTRODUCTION

The geneticist has not been content to understand the gene as it acts in the individual. His interest has grown until it has embraced problems on a population level. What is the chance that a mutant gene will survive in the population? What happens to the frequency of a gene in a population which practices random-mating? If it practices some form of inbreeding? What difference does the size of the population make? How much do different environments effect the frequency of the gene? What do the answers to such questions contribute to the theory of evolution?

The primary purpose of this research was to test Hardy's Law in experimental, laboratory populations of Drosophila melanogaster. This law[33], which is basic in any population study in genetics, states that a gene, A, with a frequency of p and its allele, a, with a frequency of q (where $p + q = 1$) will form the following proportions of genotypes in the generation following random mating:

AA		Aa		aa	
p^2	+	$2pq$	+	q^2	$= 1.$

Not only will the population just described be in equilibrium theoretically after a single generation of random mating, but, if random mating is continued, the proportions of these three genotypes will be the same in the next and in all subsequent generations. Certain basic conditions are necessary, however. The two alleles must be autosomal

and the carriers must be equally viable and equally fertile. Furthermore, the population must be infinitely large.

This type of equilibrium has been observed in a wild population of Drosophila funebris after 10,000 flies homozygous for the inversion II-2 were released. The proportions expected by Hardy's Law were observed until the time of hibernation.[27]

A Mendelian population of the type with which we will be dealing here is defined by Dobzhansky[2] as a "reproductive community of individuals which share in a common gene pool." In such a study as this one, only one locus can easily be studied at a time. The genotype of the population is a function of the genotypes of its component individuals. And yet, the rules which govern the genetic structure of the population differ from those which determine the genetics of individuals.[1]

The theories involved in any population genetics are applicable to a population of any species, plant or animal, as long as the basic suppositions inherent in the theory are met by the population in question. Dr. H. R. Hunt suggested that a laboratory population under carefully controlled conditions might serve as an interesting, practical test for Hardy's Law. Since Drosophila melanogaster is so well understood genetically, has such a short time for development, produces large numbers of offspring and is easily raised in the laboratory, this fruit fly was chosen as the species to be used in this study.

THE PROBLEM

THE PROBLEM

Can Hardy's Law be demonstrated in a laboratory population of Drosophila melanogaster? Homozygous red-eyed individuals of this species were crossed with sepia-eyed flies, and each generation that descended from this cross was allowed to breed at random in glass cages. The purpose of the experiment was to determine whether the frequencies of sepia-eyed flies from generation to generation were the frequencies theoretically expected from Hardy's Law.

APPARATUS AND METHODS

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The population cages used in this experiment were of very simple construction. They consisted of a wooden platform upon which was built a smaller, box-like cage. The extension of the platform beyond the dimensions of the cage made the cage easier to handle. This box-like cage was completely of glass except for the wooden floor and the wooden frame which supported the glass sides and top. The cage was opened by sliding upward the sheet of glass which formed one end of the cage. Four such cages were used in the entire experiment. The first two, designated A and B, were 9.5 inches wide, 15.5 inches long, and 10.0 inches in height. The other two cages, C and D, were 16.0 inches wide, 24.0 inches long and 12.0 inches high.

These cages were kept at all times in a constant temperature room at 26.5°C. (± 1.0° C). The shades in the room were kept drawn so that the room was in a semi-dark condition during the day and completely dark at night. Throughout the experiment, the medium provided for the flies was of the cornmeal-molasses-agar type. The formula for the medium was as follows:

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

The stocks of Drosophila melanogaster used here were obtained from the Biological Supply House in Chicago. The genes selected for study were those for sepia eye color and its wild type allele. Sepia is completely recessive and may be described as a deep, translucent pink eye color in freshly hatched flies which darkens as the fly ages to a very dark purplish black. It is a highly useful gene for experimentation because there is little fluctuation in the character and it is quite easily distinguished from the wild type eye color even in newly hatched flies. It is located on the third chromosome at a locus of 26.0.[6] The mutation of the wild type of sepia is quite rare although it has been reported a few times since its original discovery.[7]

In the first series of experiments with cages A and B, half pint milk bottles filled to a depth of 30mm with the medium were used in the cages. A strip of paper toweling was placed in the medium to provide a place for the larva to crawl when they were ready to pupate. The initial crosses between wild and sepia-eyed flies were made in the half-pint bottles outside the cages. When the F_1 pupae appeared, the parents were removed and five bottles of this type were placed in each cage together with ten bottles containing fresh medium. All the bottles in the cages were without stoppers. The F_1s were allowed to hatch out in the cage and to lay their eggs. When the F_2 pupae appeared in the newer medium bottles, the adult insects and the five original bottles were removed from the cage. The F_1 flies were then discarded.

This removal was accomplished in the following manner. The cage was placed in a completely darkened room and a goose-neck lamp, with its

light directed over the top of the cage at the end farthest from the opening, was placed near the cage. In about ten minutes time, most of the flies had gathered at the top of the cage in the area where the light was the strongest, since these insects are positively phototropic and negatively geotropic.[44] The cage was then opened by raising the end glass piece just enough to reach in and remove the bottles, each of which was plugged with a sterile cotton plug as it was lifted from the cage. This method of removal proved fairly efficient. Some flies were lost and although the exact number is unknown, I would estimate that 5% of the flies in the cage was the maximum number lost at any time. The average loss would be much less than that. After all the bottles had been removed, cotton wadding, soaked in ether, was placed in the cage which was then tightly closed again. When the insects had succumbed to the ether, they were gently swept out with a soft brush. The cage was then carefully cleaned with a detergent and dried.

The bottles from which the F_1's hatched were discarded. Half of the bottles (5) containing the F_2 pupae were replaced in the cage along with ten bottles of fresh media and the plugs were withdrawn. The other five F_2 bottles were kept for counting purposes. After two days the F_2 would begin to hatch; those in the cage were allowed to deposit their eggs and those in the remaining bottles were counted and the number of sepia individuals carefully noted. The two groups were assumed to have the same gene frequencies.

This cycle was repeated through the F_7 generation for the two separate cages A and B. The resultant data for experiments I and II are recorded in Tables I and II.

The results obtained by the above method were too erratic to analyze. Since the percentages of sepia varied greatly from bottle to bottle in the same generation and in the same cage, it was assumed that the sampling technique was at fault. This variation was greater by far than could be accounted for by the law of change alone. To test this hypothesis, the adults in the cages A and B of the F_7 generation, when they had laid their eggs were removed as usual but this time they were counted. This cage count was compared with the bottle count for the same cage in the same generation and a discrepancy of 4.75% and of 12.27% in the two counts was found for cages A and B respectively.

As a result, the method was modified so that no bottles were removed for counting purposes. Rather, the adults were all allowed to hatch in the cage, allowed to lay their eggs and were then removed by the method described above and all of them were counted. At the same time, the bottles (10) from which the adults had hatched were discarded, the bottles (10) in which they had laid their eggs for the next generation were left in the cage and were arranged alternately with the ten new bottles of medium added at this time. This method of separating the generations, removing and counting the flies, and of supplying new media was used in all the following phases of this experiment, whether the media was supplied in bottles or in petri dishes.

The results of generations seven through thirteen for experiments III and IV are summed up in Table III. At this time, the two cages A and B became contaminated with white-eyed flies and the experiment was discontinued.

There seemed to be a congregation of flies of one of the two types around the mouths of some of the bottles. In an attempt to remedy this, petri dishes were substituted for the bottles and used in all subsequent phases of the experiment with the cages. Eight petri dishes, 100mm in diameter, were used in each of the two cages A and B in experiments we shall term V and VI. The petri dishes were filled to a depth of about 10mm with the medium and a strip of the paper toweling was added to each dish. The four dishes which had already produced flies were removed in each generation in the same manner as the bottles. Experiments V and VI did not begin as a straight F_2 cross, but rather, different percents of sepia and wild type flies were introduced into the cages to start the populations. The data obtained from these crosses, generation one through fourteen in Cage A (experiment V) and one through eight in cage B (experiment VI) are recorded in Tables IV and V.

The larger cages C and D were begun with populations of true F_1s. These cages each held ten petri dishes 150mm in diameter, five of which were removed in each generation in the manner described previously. These dishes were filled with medium to a depth of about 15mm and a paper towel strip added to the top. The five plates containing the larva and pupa of the next generation were arranged between the five dishes containing the fresh medium. The data for the fourteen generations raised in each of these cages (experiments VII and VIII) will be found in Tables VI and VII.

Throughout this series of experiments, the cages were carefully cleaned between each generation in order to prevent the growth of molds.

The bottles were plugged and the petri dishes covered during these exchanges to avoid contamination by other flies or by molds.

As the experiment progressed, it appeared that selection was modifying the expected ratios. It occurred to me that this might be due to the selection of mates. Three tests were planned to determine this. The virgin females and the males used throughout this experiment were all 46 hours ($\pm$ 2 hours) of age. In the first case, five virgin sepia females were placed in a bottle with five sepia males and five wild males for a period of four hours. The females were then removed and put in separate bottles so that the type of offspring she produced would show whether she had been fertilized by a sepia male, a wild male, both, or in the case of no offspring, neither. This was done with a total of seventy-five sepia females and an equal number each of the two types of males. Possible mate selection for the opposite sex was also studied. In the second case, five virgin sepia females and five virgin wild females were placed in a bottle with five wild males for a period of four hours. The females were then separated and records kept of which type of female was most often fertilized. A total of eighty females was used here, forty of each type. Lastly, the experiment was repeated with the two types of females and five sepia males, and again involved the use of eighty females. The results of these crosses appear in Table VIII.

The relative reproductive ability of each of the stocks and various crosses between them when not in competition with other kinds was also studied in relation to this problem. The males and virgin females used

here were also 46 hours (± 2 hours) of age. In each case, five males and five females were placed together in a half-pint bottle containing medium. Each type of cross involved the use of 100 males and 100 females or twenty bottles. A total of five types of crosses were made, wild x wild, sepia x sepia, wild male x sepia female, sepia male x wild female, and heterozygote x heterozygote. The parents were removed from the bottle when the pupa of the next generation began to appear. The average total number of offspring produced in the five days after the appearance of the first adults for each of the types of crosses is tabulated in Table VIII.

DATA AND OBSERVATIONS

DATA AND OBSERVATIONS

The percentages of sepia in each generation in the eight populations studied is the basic material in this research. The population number is the actual number of flies counted and consists probably of a minimum of 95% of the actual population and in most cases a greater percentage than that. Male and female differences in respect to the frequency of the sepia phenotype have been recorded in all except the first two experiments.

Tables I and II give the percentages of sepia flies in the first seven generations in experiments I and II in which half the bottles containing the next generation of insects were removed from the cages and the hatching offspring counted. This sample was assumed to be like that of the bottles left in the cage to produce the succeeding generation. The number of flies and the percentages of sepia are given for each of the five bottles in each generation, and, in addition, the total percentage for that generation. The numbers of flies in these bottles averaged over 200, so that the populations in each generation usually numbered over a thousand. The percentages varied so much from bottle to bottle that the erratic results obtained over the seven generations are probably due to the random selection of the bottles which were removed from the cage for purposes of counting. The over-all data obtained here were useless in the application of Hardy's Law, but the widely varying bottle counts were of value later in the explanation of

the phenomena occurring in later populations. As has been explained, these results were responsible for the discovery of the faulty sampling technique, which was then discarded.

Table III provides the percentages of sepia obtained in experiments III and IV, which were continuations of the two preceding experiments after the flies were counted upon their removal from the cage, as was explained in the foregoing section. In cage A there was a decline from about 14.5% in the seventh and eighth generations to about 7.7% in the last three generations. In cage B the decline was more severe, falling from a high of 43.32% in the seventh generation to 7.18% in the thirteenth generation. The environmental conditions of the two cages were similar and the experiments were run simultaneously. Hence, the data obtained in the two may well be considered together. Some type of equilibrium seems to be reached in experiment III at about 7.5% and this same percentage was reached in experiment IV.

The results of the crosses begun with smaller proportions of the sepia insects than would be obtained from a direct F_2 generation are recorded in Tables IV and V. These two experiments, V and VI, showed a rise from 10.46% and 16.20% in the second generation to 19.35% and 23.12% in the third generation for cages A and B respectively. In cage A generations three through thirteen show an average of 18.72% with the greatest deviations being 16.01% in generation eleven and 21.41% in generation seven. The fourteenth generation showing 27.51% of the sepia phenotype is not consistent with the remaining portions of the data, and any uncontrolled conditions which might have caused such an increase is

unknown. In cage B, the average of generations three through eight is 20.76%, with a high of 23.70% and a low of 17.72%. In both cages a condition resembling equilibrium is evident at values that are close for the two cages and which is higher than the initial frequencies for the populations.

The largest populations which were observed were contained in cages C and D in experiments VII and VIII. The data which are recorded in Tables VI and VII show an initial rise in the F_3 generation. Thereafter, there is a decline in cage C to 11.36% and a subsequent rise to an average value of 17.44% in the last three generations. In cage D there is also a decline following the initial rise, which is followed by another rise. The last seven generations again suggest an equilibrium at an average of 22.68% with the greatest deviations being 24.41% and 21.30%, which is a relatively low range of variability.

In most of the populations, a condition resembling some type of equilibrium was established. With the use of bottled medium, this equilibrium was reached at about 7.5% for the experiments III ~~and IV~~. In experiments V, VI, VII, VIII, this value varied from 17.44% in cage C, 18.72% in cage A, 20.76% in cage B, to 22.68% in cage D. In the populations of cages A and B this value was above the initial value of the population, below the initial value in cage C, and about equal to the initial value in cage D.

In all except experiments I and II, separate counts were kept for the males and for the females. In all, sixty-one generations from six experiments were counted with the relation between the sexes in mind.

x^2 was significant (at the 5% level) in four cases and highly significant (at the 1% level) in four other cases. Since the percentage of recessives favored the males in exactly half of these eight cases and the females in half, these data as a whole were not assumed to have great significance, and calculations were based on the total percents obtained by combining the males and females.

One important difference was noted between the cages in which bottles of media were used in comparison with those in which the petri dishes were employed. The amount of moisture which condensed on the sides of the cages containing the petri dishes was much greater than those containing bottles. During the time in which the medium was being autoclaved, the bottles were stoppered with cotton plugs which prevented the entrance of much moisture. The petri dishes were covered with their glass covers and the condensation of moisture inside these dishes was considerable during the cooling period following autoclaving. The media supplied in the two cases differ in moisture content and consequently the humidity within the cages was effected. When the bottles were used, no moisture was evident on the sides of the cages, whereas, the use of the wetter medium in the petri dishes caused a clouding of the glass portions of the cage.

In each case, the experiments were begun with a fairly large number of flies. Nevertheless, the growth from this point when considered generation by generation typically follows the latter portion of Pearl's[48] population curve. That is, it grew at an accelerating rate until a maximum was reached in each generation which was dependent upon the

density of population which could be supported in the available environment. Thereafter, the growth between generations was ever less until it was no longer perceptible. In such populations of Drosophila as these, if the temperature is constant, the available food supply has been shown to be the most important limiting factor of the population.[34] This would appear to be so here also, since the competition in the larval stages was very severe. If the maximum population number which occurs continuously for a few generations is roughly estimated in round numbers and the average area and volume of available media are calculated the following correlation table may be set up:

Experiment	Estimated Maximum Size of Population A	Area of Media sq. cm. B	Volume of Media cu. cm. C
III	1800	565	1693
IV	2100	565	1693
V	1300	628	628
VI	1800	628	628
VII	4500	1767	2651
VIII	4700	1767	2651

By using the formula $r_{AB} = \frac{6\,AB}{6\,A\;6\,B}$, r_{AB} is equal to .9776 and r_{AC} is .8999. These correlations are so large that the maximum population size must be dependent to a large degree on the available food material, which is what would be expected.

Table VIII combines the results of the two collateral experiments. The first involves the testing of mate selection. The data are limited but they indicate that the two types of males are about equally competent in mating and that the sepia female shows little preference for either

type of male under the conditions tested. The females listed under the section unknown were either lost in the last transfer or became stuck in the medium before eggs had been laid. The last portion of the table lists the average number of offspring produced by ten parents (five males and five females) for the twenty bottles tested in each of the five crosses. These figures are a rough estimate of the reproductive abilities of these types of crosses under more optimal conditions than those found in the cages, since competition during the larva stage was much less in the bottles.

TABLE I

EXPERIMENT I: PERCENTAGES OF SEPIA FLIES FROM BOTTLE COUNTS IN CAGE A

Generation	Number of Offspring Each Bottle	Percentages Of Sepias in Each Bottle	Total Number Offspring	Total Number of Sepias	Total Percentages Of Sepias
F_2	289	25.61	1147	254	22.14
	211	19.43			
	274	19.34			
	139	24.46			
	234	22.22			
F_3	140	42.86	965	313	32.44
	187	43.85			
	192	30.73			
	223	29.15			
	223	21.08			
F_4	186	4.84	1179	104	8.82
	330	6.67			
	200	5.00			
	248	15.32			
	215	11.63			
F_5	295	5.76	1284	144	11.21
	310	13.55			
	258	13.57			
	228	11.84			
	193	11.92			
F_6	222	30.18	1149	260	22.63
	173	26.59			
	238	15.13			
	213	28.64			
	303	16.50			
F_7	291	25.77	1348	257	19.07
	242	9.50			
	286	19.58			
	242	21.07			
	287	18.12			

TABLE II

EXPERIMENT II: PERCENTAGES OF SEPIA FLIES FROM BOTTLE COUNTS IN CAGE B

Generation	Number of Offspring Each Bottle	Percentages Of Sepias in Each Bottle	Total Number Offspring	Total Number of Sepias	Total Percentages Of Sepias
F_2	261	24.52	1044	222	21.26
	220	16.82			
	210	16.67			
	186	23.12			
	167	25.75			
F_3	235	27.23	1091	290	26.58
	194	20.10			
	220	27.73			
	187	34.76			
	255	23.92			
F_4	266	52.26	1059	479	45.23
	163	41.72			
	124	9.68			
	302	46.36			
	204	58.82			
F_5	324	9.57	1047	205	19.58
	229	44.54			
	184	18.48			
	133	16.54			
	177	9.04			
F_6	242	28.10	1054	287	27.23
	274	21.53			
	170	15.88			
	173	49.71			
	195	24.10			
F_7	275	32.36	1240	385	31.05
	210	40.48			
	260	16.54			
	228	23.25			
	267	43.07			

TABLE III

EXPERIMENTS III AND IV: PERCENTAGES OF SEPIA FLIES FROM CAGE COUNTS IN CAGES A AND B

Generation	Percentage Of Sepia Males	Percentage Of Sepia Females	x^2 of Sex Differences	Total No. of Flies	Total No. of Sepias	Total Percentages of Sepias
			CAGE A			
F_7	14.46	14.08	.020	789	113	14.32
F_8	14.97	14.85	.004	1428	213	14.92
F_9	13.83	9.07	8.840	1600	185	11.56
F_{10}	8.82	8.37	.128	2074	179	8.63
F_{11}	5.75	6.00	.033	1176	69	5.87
F_{12}	6.02	9.37	5.501	1382	105	7.60
F_{13}	7.46	8.70	.525	1040	83	7.98
F_{14}	7.97	7.03	.413	1323	100	7.56
			CAGE B			
F_7	41.82	45.08	1.262	1175	509	43.32
F_8	31.47	30.93	.064	1875	585	31.20
F_9	22.19	20.67	.641	1871	401	21.43
F_{10}	17.95	15.62	2.120	2183	367	16.81
F_{11}	13.59	15.52	.944	1265	184	14.55
F_{12}	8.77	12.12	6.498	2151	221	10.27
F_{13}	7.16	7.19	.000	1867	137	7.18

TABLE IV

EXPERIMENT V: PERCENTAGES OF SEPIA FLIES FROM CAGE COUNTS IN CAGE A

Generation	Percentage Of Sepia Males	Percentage Of Sepia Females	x^2 of Sex Differences	Total No. of Flies	Total No. of Sepias	Total Percentages of Sepias
2	12.76	7.63	3.663	526	55	10.46
3	21.11	14.70	6.486	124	24	19.35
4	21.05	16.33	.429	125	24	19.20
5	17.55	25.76	2.079	254	50	19.68
6	17.20	17.24	.000	302	52	17.22
7	20.64	22.76	.210	341	73	21.41
8	17.44	15.79	.446	911	152	16.68
9	19.94	18.31	.519	1233	237	19.22
10	18.49	20.51	.567	887	172	19.39
11	15.42	16.76	.260	787	126	16.01
12	18.05	19.23	.745	913	170	18.62
13	19.33	19.02	.229	1297	249	19.20
14	28.57	26.33	.847	1352	372	27.51

TABLE V

EXPERIMENT VI: PERCENTAGES OF SEPIA FLIES FROM CAGE COUNTS IN CAGE B

Generation	Percentage Of Sepia Males	Percentage Of Sepia Females	x^2 of Sex Differences	Total No. of Flies	Total No. of Sepias	Total Percentages of Sepias
2	16.39	16.01	.064	2352	381	16.20
3	25.54	21.25	3.250	1276	295	23.12
4	25.15	22.39	1.500	1426	338	23.70
5	17.84	21.54	3.831	1828	365	19.97
6	16.75	18.34	.749	1732	307	17.72
7	17.22	22.11	7.232	1964	392	19.96
8	22.77	17.41	8.008	1792	360	20.09

TABLE VI

EXPERIMENT VII: PERCENTAGES OF SEPIA FLIES FROM CAGE COUNTS IN CAGE C

Generation	Percentage Of Sepia Males	Percentage Of Sepia Females	x^2 of Sex Differences	Total No. of Flies	Total No. of Sepias	Total Percentages of Sepias
F_2	25.54	19.79	5.352	1136	257	22.62
F_3	30.03	28.36	.048	1447	423	29.23
F_4	18.36	18.00	.036	1677	305	18.19
F_5	16.88	16.98	.003	1660	281	16.93
F_6	13.23	12.80	.063	1608	210	13.06
F_7	11.45	11.27	.053	3979	452	11.36
F_8	15.46	17.54	1.777	2257	372	16.48
F_9	13.67	13.87	.030	3556	490	13.78
F_{10}	13.09	14.42	1.671	4476	616	13.76
F_{11}	16.37	14.99	1.622	4516	706	15.63
F_{12}	16.23	19.56	8.357	4454	801	17.98
F_{13}	16.98	17.18	.030	4225	722	17.09
F_{14}	18.21	16.31	2.260	3549	612	17.24

TABLE VII

EXPERIMENT VIII: PERCENTAGES OF SEPIA FLIES FROM CAGE COUNTS IN CAGE D

Generation	Percentage Of Sepia Males	Percentage Of Sepia Females	x^2 of Sex Differences	Total No. of Flies	Total No. of Sepias	Total Percentages of Sepias
F_2	23.79	20.75	1.181	886	197	22.23
F_3	23.35	26.84	2.253	1410	352	24.96
F_4	20.20	20.63	.031	1079	220	20.39
F_5	16.56	19.16	1.785	1577	278	17.63
F_6	15.37	15.83	.102	2511	392	15.61
F_7	12.65	12.62	.000	2557	323	12.63
F_8	22.59	22.64	.001	3011	681	22.62
F_9	21.80	24.75	3.822	3119	725	23.24
F_{10}	20.60	22.06	1.086	3429	732	21.35
F_{11}	21.58	21.05	.183	4432	944	21.30
F_{12}	24.80	24.00	.402	4708	1148	24.38
F_{13}	21.24	21.68	.117	4256	914	21.48
F_{14}	25.08	23.81	1.027	4723	1153	24.41

TABLE VIII

PART I: MATE SELECTION

		Sepia Females	Wild Females
Cross: Sepia female x (sepia male and wild male)			
	Fertilized by sepia male	27	
	Fertilized by wild male	23	
	Fertilized by both males	13	
	Fertilized by neither	10	
	Unknown	2	
	Total	75	
Cross: (Sepia female and wild female) x sepia male			
	Fertilized by sepia male	24	25
	Not fertilized	15	14
	Unknown	1	1
	Totals	40	40
Cross: (Sepia female and wild female) x wild male			
	Fertilized by wild male	26	25
	Not fertilized	13	13
	Unknown	1	2
	Totals	40	40

PART II: REPRODUCTIVE ABILITY

		Average Number Offspring For Twenty Bottles
Cross:	Heterozygote x heterozygote	248
	Wild x wild	243
	Sepia x sepia	254
	Wild male x sepia female	261
	Sepia male x wild female	256

DISCUSSION

DISCUSSION

The main objective of this research was to test the validity of Hardy's Law in controlled populations of Drosophila melanogaster. Even as the study was just beginning, however, there was evidence that the ratios expected by Hardy's Law were being modified in some way. The numbers were quite large and theoretically, deviations from the expected ratios should not have been great. However, in experiments I, II, VII, and VIII, each of which was begun as a simple F_1 cross, the F_2 ratios in each case were less than the 25% theoretically expected according to this law. If the F_2's of these experiments are taken together, x^2 is equal to 19.155 which is a deviation from the expected which is highly significant. This evidence did not, in any sense, mean that Hardy's Law was erroneous and that the failure of these laboratory populations to follow it in any way disproved the law. Rather, it would appear that the expectations were too naive and that one or more of the basic conditions imposed upon Hardy's Law to make it valid were not being met.

A natural population is not a static entity, but a dynamic one which undergoes constant changes,[2] and the proportions expected by Hardy's Law are constantly deviating due to certain pressures. Which type or types of pressures then might be acting on these more carefully buffered laboratory populations? There are several types of nonrecurrent change which might be termed accidents.[57] These might be useful in explaining a single or at most a few erratic ratios, but not an entire series of

percentages such as was obtained here which deviate from the equilibrium values of Hardy's Law. In addition, there are systematic pressures which bear constantly upon a natural population, namely, mutation, migration and selection.[55] In this particular problem the effect of mutation should be negligible and that of migration, zero. The only remaining pressure then is selection, which is the most complex in many ways because so many variables are concerned with it. There are numerous ways in which it may effect the equilibrium ratios of Hardy's Law, and many of these will be discussed here in an attempt to find an explanation for the results obtained in this research.

It is important to remember that in any phase of the discussion of selection, and especially in the application of mathematical methods to this pressure, biological facts must be reduced to a mere abstract of their real complexity.[5] That is, only one phase will be discussed at any one time, but perhaps not one variable, but a combination of several are acting simultaneously to produce the selection phenomenon as it is observed here.

Possible Modes Of Action Of The Selection Pressure

Natural selection alters the frequencies of the genes in the next generation due to the disproportionate contribution of the carriers of the different genotypes in the preceding generation. Some of the genotypes are adaptively incompetent and may be eliminated or reduced by natural selection. Other genotypes possess optimal fitness in certain

environments, and if these environments recur frequently, these genotypes may become lasting components of the population.[1]

As we have observed in this research also, the visible effect of selection is upon phenotype. In this case, it is upon the sepia phenotype. The degree of correspondence between genotype and phenotype is fundamental in determining the permanent responses of the population to the selection pressure.[43]

Nor can we even assume that the selection rate for a single gene such as sepia is constant. There are conditions under which one gene has a selective advantage only until a certain gene-ratio is established while for higher ratios it is at a disadvantage. In such cases the gene ratio will be most stable at this limiting value for the selection and this value will tend to be restored whenever it is disturbed from either direction.[3] Neither is the adaptive value of a type necessarily proportional to its survival value at all the developmental stages of the organism. In some cases it has been shown that some types which show relatively higher mortalities than other types between the egg and adult stages proved nevertheless to be adaptively superior to the latter in the adult stage.[20]

Selection may be complete. That is, it may involve a lethal gene, so that one or the other of the genotypes is completely eliminated every generation.[4] This could not have occurred in this experiment, however, because all genotypes were present in the population and an equilibrium was reached at the approximate points already noted. Or selection may be partial, so that only a proportion of certain genotypes are eliminated

in each generation. This type of selection is most commonly against the homozygous recessives, although not necessarily so. If selection is partial and against the recessive gene in the homozygous state only, the frequency of this gene in the next generation is

$$r_{n+1} = \frac{r_n(1 - sr_n)}{1 - sr_n^2}$$

where s equals the percent of recessives rejected and r equals the frequency of the recessive gene. This type of selection is characterized by a constant decline in the recessive phenotype in every generation so that after an infinite number of generations the gene should be eliminated, or nearly so.[4] Adverse selection against a recessive gene is most effective when the homozygous recessives make up a fairly large proportion of the population. As the proportion of homozygous recessives decreases, the effectiveness of the adverse selection also decreases but at a much slower rate.[11]

A decline of this sort has been observed by several workers. L'Héritier and Teissier[35] found that in a laboratory population of D. melanogaster, the gene "bar" with an initial frequency of .999 was almost completely replaced in 150 days by the wild gene when a wild fly accidently contaminated the "bar" population. In later experiments, at the end of approximately 600 days, the frequency of the "bar" gene had been reduced to .0037 and .0105 in each of two such populations.[36] These same two workers aided by Neefs[37] found that the gene for vestigal wing was selected against in similar populations. If a breeze blew through

the cage, however, the vestigal gene was favored. Although these experiments were not carried on long enough to determine whether complete elimination would take place, these geneticists believe that neither "bar" nor vestigal would be eliminated entirely from the population, but that an equilibrium condition might be reached for a rather low frequency of the mutant gene. Gordon[32] released a population of 36,000 individuals of D. melanogaster carrying the gene for ebony with a frequency of .5. This species is not endemic in England. After 120 days, the frequency of the ebony gene had been reduced to .1. This experiment could not be continued because cold weather wiped out the entire population.

If the type of selection just described was acting in our sepia-wild populations, a constant decrease in the frequency of the sepia phenotype should have occurred. Such was not the case. A single factor may be in stable equilibrium under selection if the heterozygote has a selective advantage over both homozygotes. An inspection of the equilibrium values which have been obtained in the sepia-wild populations indicates that such is probably the type of equilibrium which has occurred in this problem.

Actually, a type of equilibrium will be obtained if the heterozygote is either better or worse adapted than the two homozygotes. Fisher[3] believes that the two cases will not exist equally frequently, however. If the heterozygote is at a disadvantage, the equilibrium is unstable, for if Δq equals 0, then $\hat{q}$ (at the equilibrium condition) is one-half, zero or one. At $\hat{q}$ equals one-half, complete or partial elimination of

the heterozygote will not change the gene frequency in the population. Therefore, random mating will restore the original zygotic proportions in the succeeding generation. But if $\hat{q}$ equals any value less than one-half, selection diminishes the frequency of the rarer allele in each generation until q becomes zero. And the equilibrium at one-half is always unstable and if lost, cannot be restored.[5]

However, if the heterozygote is better adapted, the equilibrium is more stable and will tend to persist until this stability is upset. Equilibrium will be restored once it is disturbed. At this equilibrium position, the adaptiveness of the entire population is enhanced at the price of the production of some less well adapted individuals. In this case of balanced polymorphism the average fitness of the individual in the population will be greatest when the equilibrium condition is reached.[2] If the adaptive value of the heterozygote is taken as unity then those of the homozygotes are (1-s) and (1-S) respectively where s is the selection coefficient against the homozygous recessives and S against the homozygous dominants. The frequency of the recessive gene, q, at which equilibrium is established is

$$\hat{q} = \frac{S}{(s + S)}$$

and the frequency of the dominate gene, p, is

$$\hat{p} = \frac{s}{(s + S)} \quad .19$$

If p and q are the frequencies of the dominant and recessive genes in any generation, then the frequency of the recessive gene in the next

generation becomes

$$\frac{q(1 - sq)}{1 - Sp^2 - sq^2}$$

and the amount of change in q per generation is

$$\Delta q = \frac{pq(Sp - sq)}{1 - Sp^2 - sq^2} .$$

At equilibrium Δq is equal to zero and the gene frequency ratio, $\hat{u}$, at this point is

$$\hat{u} = \frac{p}{q} = \frac{s}{S} .$$ [5]

It should be noted that in the case of the superior adaptive value of the heterozygote, the equilibrium values of a gene frequency are independent of the initial gene frequency of the population and are completely determined by the selection coefficients of the two homozygous genotypes. This equilibrium condition is a stable one and regardless of the initial frequency, it will be eventually reached since these equilibrium values of the gene frequency give the maximum adaptive value of the population as a whole.[5] This phenomenon may be observed in the sepia-wild populations in this study from experiments V, VI, VII, VIII which may be considered together since the equilibrium values of them all are very close. In experiments V and VI, the equilibrium values, about 19% and 21% respectively, are above the initial values. But in experiment VII it is below the initial value and about equal to the beginning value in VIII. This condition lends considerable evidence to the supposition that the populations in question show a superior adaptive value of the heterozygous types over the homozygotes.

If the proportions of recessives, heterozygotes and dominants are R, H, and D respectively, then

$$R_n = \frac{(1 - s)r_n^2}{P} \quad , \quad H_n = \frac{2r_n(1 - r_n)}{P} \quad ,$$

and

$$D_n = \frac{(1 - S)(1 - r_n)^2}{P}$$

where r equals the frequency of the recessive gene, S and s are the selection coefficients, and $P = 1 - sr_n^2 - S + 2Sr_n - Sr_n^2$. [4]

The explanation of the superiority of the heterozygote seems most adequately to explain the results obtained in our sepia-wild populations, since in each case an equilibrium was established which seemed to be independent of the initial values. The results obtained from the various populations may be subdivided into three groups. The first group would be composed of experiments I and II in which the counts were made from bottles removed from the cages rather than directly from the cages themselves. This first group which proved only to reveal the weakness in the sampling technique need not be considered further in this analysis. The second group would include experiments III and IV since they were carried on simultaneously and in both cases medium was provided in half-pint bottles. The equilibria reached in III and perhaps approached in IV were at the same level, although the initial frequencies differed quite markedly from one another.

Experiments V, VI, VII, VIII compose the last group. Although the population size in the first two cases differs from that in the last two, it would seem that the similar results would warrant their consideration

as a whole. The equilibrium points of them all vary within rather small limits. Relations to the initial values have been discussed.

In this particular analysis it has been found that the formulas just mentioned in connection with heterozygote superiority are practically useless since they all involve knowledge of the actual gene frequencies, p and q. Sepia is completely recessive, so the frequencies of the homozygous wild and heterozygotes cannot be determined.

If the populations had not shown selection, but had been in the equilibrium described by Hardy's Law, then the square root of the frequency of the homozygous recessive genotype would equal the frequency of the recessive gene. In our F_2 populations, the theoretical distribution of the genotypes according to this law, would be .25 aa, .50 Aa, and .25 AA. The genotypes AA and aa are apparently selected against. The proportions of AA and aa in the population would, therefore, decline and the frequency of the Aa genotype would increase proportionately. Obviously, the square root of the frequency of aa in this case would be less than .5, and the same would be true for the square root of the frequency of AA. Therefore, the sums of the square roots of the percentages of AA and aa individuals would be less than one. Consequently, since this reasoning is true in all generations, it is evident that the square root of the frequency of the aa genotype is less than q, which is the actual value of the gene a. Since p and q are unknown and cannot be calculated, the selection coefficients and adaptive values of the three genotypes cannot be determined. Moreover, we cannot even be certain that S and s are stable values. It is quite probable that they

might vary with the population density and with the frequency of the recessive gene in the total population.

In addition, it is impossible even to state whether the homozygous wild or the homozygous recessives had the higher adaptive value. There is, then, only one relationship that is known, and that is that the equilibrium value of q is greater than the value of the square root of the frequency of the sepia phenotype at the equilibrium condition. If the square root of the average frequencies of the sepia phenotype at this stable condition as they have already been stated are taken, $\hat{q}$ is greater than the approximate values of .274 for experiment III, .432 for V, .456 for VI, .418 for VII, and .476 in VIII.

The type of equilibrium observed here has been found rather frequently by a number of investigators. The superiority of the heterozygote has been shown in the experimental laboratory populations of Reed and Reed,[50] who studied populations of D. melanogaster in which the homozygotic condition for an inversion of the X chromosome was almost completely lethal when in severe competition with the wild type. However, the inversion flourished in the heterozygous condition so that it was not eliminated from the population. An equilibrium was quickly established with 28% homozygous females, 28% hemizygous normal males, 42% heterozygous females and 2% hemizygous inversion males. This shows that the heterozygous female had a strong selective advantage over the homozygous types. Dobzhansky[20] found an equilibrium of a third chromosome inversion in two populations of D. pseudoobscura at 79% and 53% respectively for the inversion in which the original breeders were

obtained from two different natural populations. This same type of equilibrium was found by Cunha[10] in regard to the trait for light and dark abdomen color in Brazilian populations of D. polymorpha with survival values of .56 for EE, 1.00 for Ee and .23 for ee. Freire-Maia[29] also observed a stable value for dark and light abdomen color in Brazilian populations of D. montium.

Additional details of this type of equilibrium were obtained by Kalmus[40] in populations of D. melanogaster with respect to the trait ebony. He found that at a higher temperature and higher humidity, the wild type was adaptively superior to ebony. But the ebony gene was found to be superior under the opposite conditions. However, in each of the cultures, eventually an equilibrium was approached even though the point of stability differed with the varying conditions. L'Héritier and Teissier[36] found that in their populations of ebony and wild, an equilibrium was established at about 15% of the ebony flies.

This same situation has been observed in natural populations by Dobzhansky and Levene.[24] These two workers found that the eggs laid by D. pseudoobscura are in conformity with the Hardy-Weinberg Law in the proportions of homozygotes and heterozygotes for different types of the third chromosome. But a differential mortality takes place between the egg and adult stages which favors the heterozygote.

Thus far, we have considered that the wild-sepia populations studied have had the ratios expected by Hardy's Law modified by the pressure of selection which favored the heterozygote. It would be interesting to attempt to explain what environmental factor or factors might have

contributed to this selection and in what stage of the life cycle it might act.

Period Of The Life Cycle Where Selection Exerts Its Pressure

Selection may occur at any stage in the life cycle. If there is a differential fertility of the adult flies, it may be in the number of eggs or sperm produced or in the relative survival ability of the gametes. A smaller proportion of the eggs of one genotype may hatch as has been shown to be the case in some of the other mutant stocks of Drosophila.[47] One type may be at a disadvantage in the competition in the larval stages. Perhaps one is better adapted to survive the pupa stage. The time and length of reproductive activity may vary. In the adult stage it may occur in the selection of mates. Nor is any one of these constant, but is modified by numerous environmental conditions, such as temperature, food and population density.

Mate selection has been observed in certain populations of Drosophila. Reed and Reed[51] found that natural selection favored the wild gene in laboratory populations of this insect to the extent of eliminating the gene for white eye. They discovered that the ratio of red males which succeeded in mating compared to white males was 1.00 to .75. Thus they were able to conclude that selective mating was the most important factor contributing to the decrease in the white gene. This same conclusion was reached by another worker[12] in populations of wild and yellow-white. Rendel[52] observed the courtship pattern in D. subscura of yellow males and found that it did not differ from that of normal males except that it

was longer. But the normal female resisted the advances of the yellow male. Merrell[45] in his selective mating experiments also concluded that the occurrence of non-random mating was due primarily to the behavior of the female in D. melanogaster, but that in time, practically all females of a population would be fertilized. Sturtevant[54] has also studied the problem of sexual selection in this fly. His results indicate that actual choice is not involved. But that any female willing to mate will accept any male and a male ready to mate will do so with the first female which will let him. However, if the female is not willing, the male of the more vigorous stock will have an advantage. As a result, the weaker female is most often mated with.

It was with this possibility in mind that the experiment on mate selection with the wild and sepia stocks was begun. However, the results are startlingly close for the small numbers worked with. The wild and sepia males seem to be equally successful in mating with both types of females and either of the two males seem to be acceptable to the females. The numbers are not large enough to be conclusive but they indicate that mate selection probably does not play a very important role in these sepia-wild populations, in producing the selection pressure.

In addition, the numbers of offspring produced by the different types of wild-sepia crosses indicated in Table VIII show little variability in their ability to reproduce at uncrowded, more optimal conditions. This information would cause me to believe that the reproductive capacities of the different types of crosses does not differ too much, and that under optimal conditions, about the same number of offspring

reach maturity. Moreover, differential mortality in the adult stage may be eliminated since never more than two or three dead flies were found in any cage during a generation's time. The most probable point of the action of the selection pressure is during the larval stage. The cages were supporting the maximum number of flies possible with the available food supply and the medium dishes were quite crowded with larva and the competition at this point was quite keen.

Possible Agents of Selection

Numerous environmental factors act as agents of natural selection, and many of them have been studied in Drosophila populations. Seasonal variations have been observed in the relative frequency of gene arrangements in the third and sex chromosomes in two of three populations of D. pseudoobscura obtained from localities near San Jacinto, California.[16] Three Moscow populations of D. funebris,[25] and a certain population of D. robusta[42] from Virginia also showed seasonal variations. In the latter case, the changes were significant in males only. The relative frequency of black hamsters has been reported in some regions of U.S.S.R. to undergo regular and significant changes from season to season.[31] All of these reports indicate that selection may act quickly in changing the frequencies of gene arrangements and of genes. Probably several environmental factors which change with the season cause these changes.

In D. pseudoobscura, Wright and Dobzhansky[57] have found in laboratory populations that certain of the third chromosomal arrangements are better adapted at higher temperatures and others at lower temperatures.[17] These

varying adaptive values to temperature were also found in certain chromosomal variants of the second and fourth chromosome in this same species.[23] Hovanitz[38] found that the frequency of white females in the butterfly, Colias chrysotheme are largely correlated with changes in climatic conditions. Large size seems to be similarly correlated in the English sparrow.[8] Extreme cold temperatures which caused individuals of D. funebris to hibernate was found to favor one inversion and to discriminate against another.[28]

Other environmental factors not clearly outlined serve as selection pressures on populations of D. funebris observed by Dubinin and Tiniakov.[26] These workers consistently observed higher frequencies of certain inversions in the populations of Moscow and other cities than in the rural districts nearby.

Another factor which acts as an agent of natural selection is population density. Pearl and Parker[49] have found in early studies of Drosophila that the reproductive rate per female declines as the population becomes more dense but the decrease is at a decreasing rate at the highest densities. Crowding has been seen to play an active part in some laboratory populations. Moree[46] found that the mutant gene ebony in populations of D. melanogaster was nearly as viable as wild if there were little crowding. But the viability of ebony decreased as the crowding continued and as the competition became more intense. Other workers have observed that some inversions on the second and fourth chromosomes[23] and in crosses involving three gene arrangements on the third chromosome[17] of D. pseudoobscura there was a differential viability in accord with the

population densities. Other gene arrangements of the third chromosome were little modified by these conditions. The incidence of black coat color in some hamster populations of the U.S.S.R. was found to be positively correlated with the population densities of that species.[30]

The additive effects of temperature and humidity on the frequency of ebony in Drosophila populations ~~was~~ were mentioned very early in this Discussion.

Which of these factors then seem greatest in influencing the selection observed in the wild-sepia populations of this research? Since temperature was carefully controlled, the changes were not due to any fluctuations here. However, the temperature was probably very important in determining the adaptive values of the three genotypes and therefore the actual point of equilibrium. The humidity was approximately equal in each cage throughout any one experiment, since it seemed to depend on the wetness of the medium provided. Its probable importance in producing the widely varying equilibrium points at about 7.5% in III ~~and IV~~ and between 17.44% and 22.60% in experiments V-VIII has already been mentioned. It is probably one important factor in determining this difference.

Another factor already mentioned is that of population densities. This crowding was quite severe during the larval stages, and intense competition was, no doubt, of utmost importance as a selective agent. The exact importance of any one of these factors would have to be determined experimentally, but I believe that it is safe to assume that as a group, temperature, humidity and crowding were of some importance in determining the equilibrium points in the wild-sepia populations.

The Importance Of Balanced Polymorphism As Determined By Selection Pressures

In natural populations the balanced polymorphism observed in these populations buffers the species against environmental change and at the same time it does not consume or deplete the store of hereditary variability present in the population. The total adaptive ability of the population is thus greatly enhanced.[18]

A balanced polymorphism also preserves certain of the new gene arrangements and gene mutations which arise from time to time in any population. This is important because it maintains these two most important raw materials of evolution,[13] which are present much more frequently in Drosophila populations than would be expected by casual observation.[14,39] These raw materials are then acted upon by restriction of population size, natural selection and the development of isolating mechanisms to insure the progress of evolution.[1]

The populations in this study were all fairly large except in the first generations in experiment V. If the size had continued small and a considerable number of generations had been allowed to pass, a drift away from the equilibrium values would have been expected from chance alone. In such populations the effective size of the breeding population must be taken into account[56] and the inbreeding coefficient must be determined[22] since there will be a corresponding decrease in heterozygosity due to these factors.[5] In these smaller populations, chance plays a much greater role in producing widely varying populations. This type of drift has been seen to play an important part in the differention of certain

local populations in several species of Drosophila, the amount of differentiation depending upon the effective size of the breeding population.[53,21, 41,9,15] The size of the wild-sepia populations studied here should not have allowed for any measurable amount of genetic drift. The equilibrium points in experiments V-VIII varied somewhat but within reasonable limits, with but a difference of only 5.2% between the highest in cage D and the lowest in cage C. It would be impossible to say that the conditions in the four experiments were identical although they were kept as close as possible. These existing differences in conditions alone could probably explain the 5.2% variation.

It is also apparent from the data that the equilibrium is not absolutely stable, but certain unknown factors occasionally upset it. Perhaps chance plays some part here. But in every case, the populations were restored to the equilibrium point in one or a few generations. The most difficult variation of this type to explain is in the fourteenth generation in experiment V. This last generation reached a high of 27.5% of the sepia individuals after having been at a fairly stable equilibrium for many generations before. No known accidental factor entered the experiment here, although it is not impossible that the population was affected by some factor which was not seen or understood. There is reason to believe, however, that whatever the cause, if the population had been continued, it would have returned to its equilibrium level in a few generations.

A number of questions naturally arose as the study progressed. As has been indicated already, the data would have been much more useful

if the actual gene frequencies had been known. This could have been determined very simply by progeny testing a sample of the wild females in each generation to determine the percentage which were heterozygous. Such wild females should be mated with sepia males. Then by use of the formulas already presented, the selection coefficients and adaptive values for the three genotypes could be calculated.

Other possibilities present themselves. It would have been interesting to begin populations with very high and with very low frequencies of the sepia gene. Since humidity was indicated as an important factor in setting the stable position, such a study as to its importance might be pursued further. Varying the temperature and the density of the population would help to discover what part these factors play in establishing the adaptive values of the three genotypes.

CONCLUSIONS

CONCLUSIONS

1. The wild-sepia populations studied did not follow the ratios expected by Hardy's Law but were modified by selection pressures.

2. The most likely explanation for the type of selection is in the superiority of the heterozygote over the two homozygotes, since an equilibrium was reached in each case which seemed to be independent of the initial frequency. The equilibrium values were approximately 7.5% of sepia individuals in experiment III, 18.72% in experiment V, 20.76% in experiment VI, 17.44% in experiment VII, and 22.68% in experiment VIII.

3. The equilibrium frequencies of the recessive gene are greater than the square root of the proportions of homozygous recessives and are above the approximate values of .274 in experiment III, .432 in experiment V, .456 in experiment VI, .418 in experiment VII and .476 in experiment VIII.

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