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A GENETIC STUDY OF THE AMYLASE ISOZYME POLYMORPHISM IN *DROSOPHILA MELANOGASTER*

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IN GENETICS

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By

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ABSTRACT

Many of the recently discovered genetically determined polymorphisms involving isozymes of Drosophila melanogaster have been concerned with identifying the enzyme biochemically and determining the formal genetics of the enzyme. After the survey of several enzyme systems in natural populations of Drosophila pseudoobscura by Lewontin and Hubby (1966), which indicated that isozyme polymorphisms are more frequent than previously expected, there has been greater interest in population studies of these isozymes as is shown by the recent work in Drosophila melanogaster concerning the Esterase 6 polymorphism (Yarbrough and Kojima, 1967) and the alcohol dehydrogenase polymorphism (Kojima and Tobari, 1969b).

The purpose of this study was to investigate the frequencies of the amylase isozyme alleles in several natural populations of D. melanogaster, and on the success of this investigation, to attempt to see how the polymorphism might be maintained.

The survey consisted of recently collected flies from natural populations of Texas and Wisconsin. In addition, the Odate population (collected in Japan four years ago and since kept in the laboratory) was also examined. For all surveys the frequency of the Amy^{1} allele was clearly the most common (0.80) and was followed by $Amy^{1,3}$ (about 0.12). Other less frequent alleles observed were $Amy^{2,3}$, $Amy^{1,2}$, and $Amy^{1,6}$.

Because of Amy^{1} and $Amy^{1,3}$ being consistently the most

frequent in the natural populations, the selection studies concentrated on these two alleles in the Texas and Wisconsin populations. The results of the viability and fertility experiments (for matings within and between populations) consistently demonstrated higher viability and fertility for the $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ genotype followed by $\underline{Amy}^{1}/\underline{Amy}^{1,3}$ and $\underline{Amy}^{1}/\underline{Amy}^{1}$ genotypes respectively. The results of the developmental study, which employed the normal sugar containing media and a special media containing starch instead of sugar, were less consistent; however, the $\underline{Amy}^{1}/\underline{Amy}^{1}$ genotype had the slowest developmental time with the $\underline{Amy}^{1}/\underline{Amy}^{1,3}$ and $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ genotypes being similar to each other.

From these results it is difficult to see why the Amy^{1} has such a high gene frequency in natural populations. There was some indication from one of the viability experiments that the recovery frequency of the $\underline{Amy}^{1}$ allele among progeny is higher than that of the $\underline{Amy}^{1,3}$ allele when the parents are very young, thus suggesting a prezygotic mechanism of some sort. This study, however, does not critically test for prezygotic selection and more specific tests are currently being planned.

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CHAPTER I

INTRODUCTION

Background

This rapid development in the study of genetically determined protein has largely been due to improved techniques for the separation of proteins, such as starch gel electrophoresis (Smithies, 1955; Smithies, 1959) and the introduction of specific biochemical staining techniques for various enzymes (Hunter and Markert, 1957). Enzymes which are distinguished into different molecular types but catalyze the same substrate are called isozymes (Markert and Moller, 1959). This enzyme variation for a large number of organisms is summarized in a review by Shaw (1965).

In Drosophila melanogaster, Wright (1961) reported the presence of two different esterase bands which were genetically controlled by two co-dominant alleles. These two bands were called $Est6^F$ and $Est6^S$ and were mapped at position 36.8 on the third chromosome (Wright, 1963). A different esterase locus, known as Est C, was discovered by Beckman and Johnson (1964a) on the third chromosome; however, it was not found to be closely linked to the Esterase 6 locus. Both Esterase 6 and Esterase C can each exhibit a fast band (homozygote), a slow band (homozygote), and both a fast and slow band (heterozygote). Some of the gene-enzyme systems which have been discovered in Drosophila melanogaster are: x-amylase (Kikkawa and Abe, 1960), glucose-6 phosphate

melanogaster are: α-amylase (Kikkawa and Abe, 1960), glucose-6 phosphate dehydrogenase (Young, Porter, and Childs, 1964), alcohol dehydrogenase (Johnson and Denniston, 1964; Grell, Jacobson, and Murphy, 1965; Ursprang and Leone, 1965), alkaline phosphatase in larva (Beckman and Johnson, 1964b), acid phosphatase (MacIntyre, 1966), leucine amino-peptidases A and D (Beckman and Johnson, 1964c), 6-phos-phogluconie acid dehydrogenase (Kazazian, Young, and Childs, 1965), and xanthine dehydrogenase (Glassman and Saverance, 1963).

This study will be concerned with the α-amylase isozymes of Drosophila melanogaster. The α-amylase or 3.2.11 C x-1, 4-glucan 4-glucanohydrolase hydrolysis the x-1,4 glycoside links in polysaccharides but does not hydrolyze 1-6 gly-cosidic links of a branch chain polysaccharide such as glycogen. This enzyme has been found in various animal tissues, saliva, plants, molds, and bacteria.

Genetic variations in amylase have been observed in such diverse organisms as: pigs (Ashton, 1960), cattle (Ashton, 1965), mice (Sick and Nielson, 1964), houseflies (Ogita, 1962), Drosophila virilis (Kikkawa, 1963), Drosophila simulans (Kikkawa, 1965), maize (Scandalious, 1965), barley (Fryden-berg and Nielson, 1965), and man (Kamaryt and Laxova, 1965). All of the above examples involve an observable electrophore-tic change which is probably inherited from two or three alleles at a single locus.

The α-amylases in Drosophila melanogaster were reported by Kikkawa and Abe (1960) with the genetic variation being based upon distinct levels of activity of the enzyme in various fly strains. These activity levels were classified into strong, weak, and intermediate. This genetic variation was found to be more complicated when Kikkawa (1964) was able, by employing agar gel electrophoresis, to infer the presence of seven amylase alleles. These alleles were listed as $\underline{Amy}^{1}$, $\underline{Amy}^{1,3}$, $\underline{Amy}^{4}$, $\underline{Amy}^{1,6}$, $\underline{Amy}^{2,6}$, $\underline{Amy}^{3,6}$, and $\underline{Amy}^{4,6}$ with band number one being the fastest migrating band toward the anode followed by band numbers three, four, and six. Doane (1966), who utilized polyacrylamide gels in a disc electrophoresis apparatus, discovered an additional allele which was named $\underline{Amy}^{1,2}$, and established that the $\underline{Amy}^{4}$ stocks reported by Kikkawa also had a one band. The $\underline{Amy}^{4}$ stocks were renamed $\underline{Amy}^{1,4}$. Recently Bahn (1967) reported a $\underline{Amy}^{2,3}$ allele, which had been isolated from a Bennett population cage. Kikkawa, in 1964, observed that the amylase locus was on the second chromosome at position 78.1, and Doane also reported it on the second chromosome at 77.3 (Doane, 1965a). There are at present nine known amylase isozyme alleles with heterozygotes exhibiting a co-dominant effect with no evidence for the formation of hybrid enzymes. A diagram illustrating how some of the amylase genotypes appear is shown by Figure 2 on page 12.

Although each of the two-banded patterns appeared to be due to a single gene, there remained the possibility that two

very closely linked genes might be responsible for the two banding pattern with each gene determining a single band. This question was resolved when Bahn (1967) demonstrated that recombination occurred within some of the two-banded alleles. The linkage distance between the two genes was of the order of 0.008 map units with each gene determining a single amylase band. From this recombination data, Bahn (1967) suggested that the two genes might have arisen by duplication from the $\underline{Amy}^{1}$ gene which is presumed to be the ancestral type as it is the most common. Since different activities have been observed in some of the $\underline{Amy}^{1}$ strains (Doane, 1968), there remains the possibility that some of the Amy^{1} strains may also be either a duplication or have a closely linked modifier. It is clear that further investigation will have to be conducted to resolve this problem.

There does not appear to be either the formation of any new bands or loss of any particular bands during the onotogeny of the fly; however, the relative activity of the two bands changes during development (Doane, 1965). In general, as development from larva to adult proceeds, there is a tendency for activity to decrease in the faster migrating bands and increase in the slower migrating bands. This was especially true for $\underline{Amy}^{1,4}$ stocks with the number one band often being very weak in adults. The activity of the various amylase types varies greatly from those alleles having low activity (Amy^{1}, $Amy^{1,4}$) to those (such as $Amy^{1,6}$ and $Amy^{2,6}$) with four times this activity (Doane, 1967).

Purpose of the Present Study

The amylase alleles which Kikkawa observed were isolated from natural populations in Japan, but the frequency distribution of these alleles was not determined. So far, no information is available for the frequency distribution of each of the alleles in natural populations of Drosophila melanogaster except that, as was suggested by Kikkawa, the Amy^1 allele is the most common.

The first objective of this study is to investigate several natural populations of D. melanogaster for the frequency distributions of the amylase isozyme alleles, and on the success of this investigation, the second objective is to find out the evolutionary factors that could maintain the amylase isozyme polymorphism in this species.

MATERIALS: (A) Flies from natural populations.

1. Austin, Texas population. Approximately 176 males of Drosophila melanogaster were collected from a natural population in Austin, Texas during the period from late spring to early summer, 1967, by Dr. Denell. These sampled males were sent to Hawaii and were typed for their amylase isozyme banding patterns.

2. Madison, Wisconsin population. These flies were collected from a natural population in Madison, Wisconsin during the summer of 1967 by Dr. Hiraizumi. The sampled flies were placed into vials in order to establish independent lines with two or three pairs of sampled flies per

line. A total of sixty lines of D. melanogaster were established. These were kept in a laboratory at Madison for 1-3 generations and were then brought back to Hawaii for typing. Only one fly from each line was sampled.

3. Odate, Japan population. These flies of D. melanogaster were collected in 1963-1964 from a natural population of Odate, Japan. About 116 independent lines were established in a similar manner as was done for the Wisconsin samplings, except that each line was established with ten or more pairs of sampled flies. Since then, each of the lines has been kept in a separate culture vial.

(B) Flies from Laboratory Stocks. These mutant stocks are described by Lindsley and Grell (1967).

1. cn bw. This stock carries two recessive markers cn (cinnabar eye, 2R-57.5) and bw (brown eye, 2R-104.5). It is a very easy stock to keep and was used as a standard stock throughout this study.

2. SM-5/ cn bw. The SM-5 chromosome carries complex inversions which are excellent as a balancer for the whole second chromosome. It also carries a dominant mutant Cy (curly wings) and a recessive lethal gene. For simplicity, this chromosome will be called Cy throughout this study. Before being used in any of the present experiments, the background of this stock except for the Cy chromosome had been replaced by that of the standard cn bw stock.

3. y f:=/Y; cn bw. This stock carries a reversed

acrocentric attached-X chromosome which has the recessive markers y (yellow body, 1-0.0) and f (forked bristles 1-56.7). The background of this stock except for the attached-X chromosome had been replaced by that of the Standard cn bw stock. All of these laboratory stocks were typed numerous times for the amylase genotype and were always found to be Amy^1/Amy^1.

FOOD MEDIA

The formula of the standard culture media used in this investigation is as follows:

1 liter	Water
66 grams	Sucrose
50 grams	Brewers Yeast
18 grams	Agar
5 ml.	Propionic acid
7 ml.	methy[1] p-hydroxybenzoate

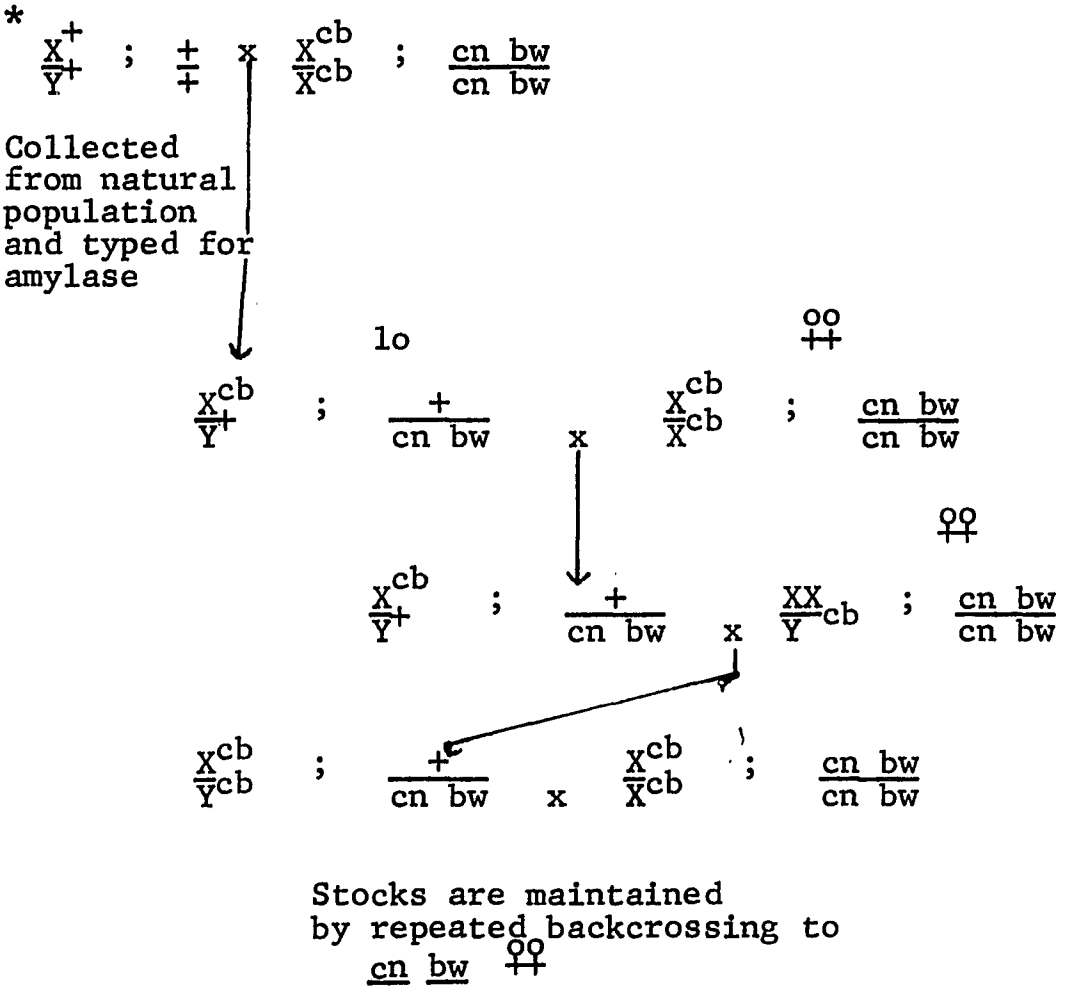
A special media, which was similar to the formula employed by Bahn (1967), was utilized in a portion of the developmental study. It is identical to the standard culture media except that thirty grams of soluble starch is substituted for the sugar. This media will be referred to simply as the starch media.

METHODS: (A) Mating scheme for making a homogeneous background.

As long as one's interest is in finding out the evolutionary factors that maintain amylase isozyme polymorpism in natural populations, it is in principle desirable to

conduct experiments under the genetic backgrounds in natural populations, but this is practically impossible.

In the present study, an attempt was made to make the genetic background other than the second chromosome (where the Amy allele is located) homogeneous, so that any significant differences in any comparisons have to be due to the differences in the second chromosome constitutions. For this purpose the second chromosomes sampled from natural populations were substituted into the standard, cn bw, stock background, as is shown in Figure 1.



* The X^+ , Y^+ , and + signs refer to X, Y, and second chromosomes taken from natural populations.

FIGURE 1. MATING SCHEME FOR SUBSTITUTING THE SECOND CHROMOSOME INTO THE STANDARD cn bw BACKGROUND.

This mating scheme replaces the X-chromosome as well as the third and fourth autosomes by repeated backcrosses of the male (+ / cn bw) progeny to virgin cn bw females. The Y-chromosome is replaced by mating the male progeny to the attached-x females. By this procedure several second chromosomes containing a particular amylase type were isolated and maintained in the homogeneous background. After several generations of backcrosses, the viability, fertility, and developmental time experiments were conducted from some of these amylase lines. The specific mating scheme of each of these experiments will be presented at the beginning of each chapter which discusses that experiment.

Electrophoresis Technique

The technique used in this investigation employed a 7% cyanogum gel in a horizontal electrophoresis setup. Cyanogum is composed of a mixture of 95 percent acrylamide and 5 percent methylenebisacrylamide (White, 1960). The electrolyte and buffer for the gel was developed by Ferguson and Wallace (1961). The electrolyte is prepared by dissolving 0.75g. of lithium hydroxide and 11.8g. of boric acid in one liter of water. The buffer in which the cyanogum is dissolved consists of nine parts of a solution containing 1.6 g. of citric acid and 4.8g. of tris (hydroxymethyl) aminomethane per liter plus one part of the electrolyte solution.

The preparation of the flies, which is quite similar to the technique employed by Johnson (1964) on starch gels,

is simple and quick. Each fly is ground with one or two drops of buffer solution in a spot plate and the moisture from the homogenate is drawn up into a small piece of Whatman 3mm filter paper. The sample is then inserted into a slit cut 3 1/4" from one end of the gel and the slit is gently pressed shut. In this manner thirty-two samples can be placed in each tray. The gel is then run for approximately two and a half hours at a current of 50 ma and 200 volts. Handi-wrap was used to prevent drying of the gel during the run and cooling is provided by either turning the air conditioner ducts towards the gel or running in the refrigerator. Afterwards, the gel is removed and placed into a 1% soluable starch solution containing a Tris-HCl buffer of pH 7.4, since Doane (1966) considers this pH optimum for this particular enzyme system. After an incubation period of at least two hours in this solution, the excess starch is rinsed off the gel and it is placed into a 0.1N solution of I_2-KI. This quickly turns the entire gel dark blue except where the amylase has broken the starch down. These appear as light blue or transparent bands against the dark blue background of the gel. The gel may be stored in the dark in a 7 percent acetic acid solution; however, it is better to read the gel immediately after staining. A diagram of some amylase genotypes is shown in Figure 2.

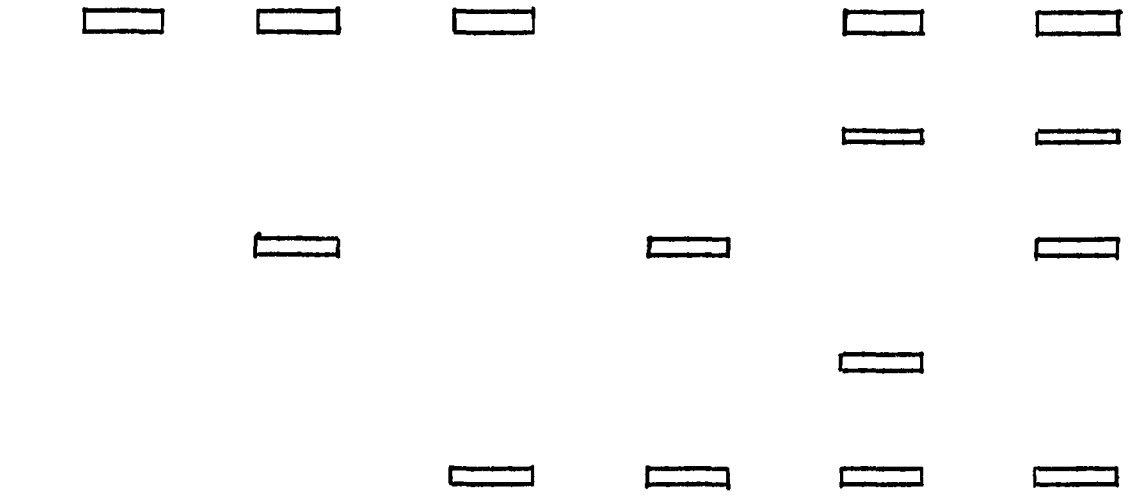


FIGURE 2. DIAGRAM OF SOME AMYLASE GENOTYPES

CHAPTER II

POPULATION SURVEY

Although samples were taken from four natural populations (Odate, Japan; Honolulu, Hawaii; Madison, Wisconsin; Austin, Texas), further studies were done on only two of these, i.e., Madison and Austin, from which sample flies were collected and were typed for amylase recently. The sample flies from the Odate population were collected during the period 1963-1964 and had since been maintained as laboratory stocks in small culture vials. The sampling from Hawaiian populations was not successful.

ODATE POPULATION

Since these lines had been maintained for a long period of time in the laboratory, they cannot be considered to provide true frequency distributions of amylase alleles in the original natural population because the environmental conditions in the laboratory would be different from those in the natural population, and this might change the original frequency distributions. It could be also expected that some of the alleles which originally existed in this population might be lost by chance fluctuations due to the small population size in each line culture vials. Yet it is interesting to investigate what kind of amylase alleles could be found among lines for the Odate population.

A total of 1,516 flies were typed for amylase from 116 separate lines. The results are summarized in Table I.

TABLE I

ODATE POPULATION SURVEY

Amylase type found in each line	Number of lines	Frequency
Amy^1 only	109	0.939
$Amy^{1,3}$ only	1	0.009
$Amy^{1,4}$ only	1	0.009
$Amy^{1,3}$ and Amy^1	2	0.017
$Amy^{1,2}$ and Amy^1	3	0.026
Total	116	1.000

From Table I, it is quite evident that $\underline{Amy}^1$ is the most common which is in agreement with Kikkawa's observation (1964). This population also contained $\underline{Amy}^{1,3}$, $\underline{Amy}^{1,2}$, and $\underline{Amy}^{1,4}$ but in much reduced frequencies. Possibly other alleles which might have existed in the original Odate population in low frequencies were lost from the line cultures, but this cannot be learned from this survey.

From the seven lines in the Odate population that contained some allele other than $\underline{Amy}^1$, two lines appeared to have become fixed for either $\underline{Amy}^{1,3}$ or $\underline{Amy}^{1,4}$. There was a total of ten flies from each line which were typed, but since neither $\underline{Amy}^1/\underline{Amy}^{1,3}$ and $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ nor $\underline{Amy}^1/\underline{Amy}^{1,4}$ and $\underline{Amy}^{1,4}/\underline{Amy}^{1,4}$ can be distinguished from each other by electrophoresis, these two lines could still contain the $\underline{Amy}^1$ allele in addition to $\underline{Amy}^{1,3}$ or $\underline{Amy}^{1,4}$.

There were two alleles segregating for the remaining five lines. The observed number of flies of each genotype and the estimated gene frequency for the $\underline{Amy}^{1,3}$ and $\underline{Amy}^{1,2}$ alleles in each segregating line is given in Table II.

TABLE II

OBSERVED PHENOTYPES IN THE FIVE SEGREGATING LINES FROM THE ODATE POPULATION

Line Number	Amy type	Number of flies	Amy type	Number of flies	Total	Gene frequency
j-3	$Amy^{1,3}$	27	Amy^{1}	44	71	$Amy^{1,3}=0.21\pm0.04$
R-4	$Amy^{1,3}$	9	Amy^{1}	1	10	$Amy^{1,3}=0.68\pm0.15$
Q-6	$Amy^{1,2}$	8	Amy^{1}	11	19	$Amy^{1,2}=0.24\pm0.07$
I-9	$Amy^{1,2}$	7	Amy^{1}	3	10	$Amy^{1,2}=0.45\pm0.13$
I-5	$Amy^{1,2}$	5	Amy^{1}	5	10	$Amy^{1,2}=0.29\pm0.11$

The interval between the period when the typings were made and when most of the lines were established was approximately four years. This would correspond to at least 100 generations (taking two generations per month--actually more than two generations). The population size in the line culture was very difficult to estimate with the number of flies transferred to the next generation fluctuating from generation to generation (2 or 3 pairs--10 pairs). In maintaining these lines, no attention was paid to the sex ratio among transferred flies. It had randomly fluctuated throughout the period and this would cause reduction in the

effective population size. A crude estimate (possibly over estimate) of the effective population size of these lines over the four year period would be ten. Let q be the initial gene frequency of one allele and $1-q$ be the gene frequency of the other allele (or alleles) at a given generation. Let N be the effective population size. Then, assuming no selection and no mutation, the probability (P_t) of two alleles to co-exist in a population after t generations is given approximately by

$$P_t = 6q(1-q)e^{-\frac{1}{2N}t} + 14\,q\,(1-q)[1-5q(1-q)]e^{-\frac{6}{2N}t} + \ldots$$

or, when t gets large

$$P_t \doteq 6q(1-q)e^{-\frac{1}{2N}t} \qquad \text{(Kimura, 1955).}$$

The probability, P_t, has its maximum value when $q = 0.5$ provided that N and t stay the same. Let q be the frequency of the Amy^1 allele in the original Odate population and let us assume $q=0.5$ to give the maximum value for P_t. Then for the Odate line culture population, we have

$$P_t \doteq (6)(0.5)(0.5)e^{-\frac{100}{20}} \doteq 0.01.$$

If the frequency of Amy^1 was greater than 0.50, the effective population size was smaller than ten, or the number of elapsed generations since the lines were established is greater than one hundred, the value of P_t would be less than 0.01. From these calculations it would be expected that at most 1% of the line cultures would contain two amylase alleles, but actually at least 5 out of 116 were found to contain two alleles. This strongly suggests that at least in the five

segregating lines a strong selection had been operating to maintain the two alleles in these line cultures.

HAWAIIAN POPULATION

The attempt to collect Drosophila melanogaster in Hawaii was largely unsuccessful. Culture bottles containing media plus live yeast were set out among rotting mountain apples and bannans in two locations in Manoa and one location in a banana grove near Kailua. Many flies were collected during the months of April and May in 1967.

These flies appeared to be Drosophila melanogaster but were actually Drosophila simulans. Hardy (1965) had reported that D. simulans was much more common in Hawaii than D. melanogaster. An examination of the male genitalia indicated that most of the flies were D. simulans, however, to be certain, several crosses were made between one male from the natural population with three to five cn bw females. From a total of 187 crosses only five were quite fertile with the males all proving to be Amy^1/Amy^1. An examination of some of the sterile or nearly sterile matings revealed the male flies to have bands corresponding to Amy^4 of D. melanogaster which is identical to the results Kikkawa (1965) obtained in an amylase study in this D. simulans, provides further evidence that the "sterile matings" were due to D. simulans. These collections were stopped completely when it was observed (fortunately before being taken to the laboratory) that one of the collections was full of mites.

TEXAS POPULATION

There were three shipments of male flies from a natural population in Austin, Texas which arrived during June, July, and August of 1967. These males were typed after each male was crossed to three or four *cn bw* virgins. Approximately half of these flies were apparently *D. simulans* since they gave no offspring when crossed with *cn bw* females. If this male was something other than Amy^1, five of the male F_1 offspring were typed in an attempt to determine the genotype of the parental male. There were, however, several cases of the $Amy^{1,3}/Amy^{?}$ ($Amy^{?}$ could be either Amy^1 or $Amy^{1,3}$) genotype where the F_1 progeny test could not be made. The results of this survey are summarized in Table III.

TABLE III

SURVEY FOR AMYLASE GENOTYPES FROM

A TEXAS POPULATION OF D. MELANOGASTER

Amy Genotype of male parent	Number of flies typed	Gene frequency	Maximum likelihood estimation
Amy^1/Amy^1	122	Amy^1 0.837±	0.020
$Amy^{1,3}/Amy^?$ *	38	$Amy^{1,3}$ 0.116±	0.020
$Amy^{1,3}/Amy^{1,2}$	1	$Amy^{1,2}$ 0.026±	0.017
$Amy^{1,2}/Amy^1$	8	$Amy^{2,3}$ 0.009±	0.006
$Amy^{2,3}/Amy^1$	3	$Amy^{1,6}$ 0.011±	0.005
$Amy^{1,6}/Amy^1$	4		
Total	176		

* $Amy^?$ would be either Amy^1 or $Amy^{1,3}$.

From the results of Table III, it is evident that Amy^1 is by far the most frequent gene in the population and $Amy^{1,3}$ was also present in a reasonably high frequency. The other three alleles were present in a low frequency.

Since many of the $Amy^{1,3}/Amy^?$ flies could not be determined for their genotype, and since the frequencies of alleles other than Amy^1 and $Amy^{1,3}$ were too low, the goodness of fit to the Hardy-Weinberg distribution was not tested.

WISCONSIN POPULATION

A total of 59 lines were examined by typing one sample fly from each line and then examining the F_1 flies if the sample parent was a type other than Amy^1. The genotypes of

all sample flies were determined. The results are given in Table IV.

TABLE IV

SURVEY FOR AMYLASE GENOTYPES FROM A WISCONSIN POPULATION OF D. MELANOGASTER

Amy Genotype of male parent	Number of flies typed	Gene frequency	
Amy^{1}/Amy^{1}	34	Amy^{1}	0.746± 0.040
$Amy^{1,3}/Amy^{1}$	9	$Amy^{1,3}$	0.119± 0.030
$Amy^{2,3}/Amy^{1}$	7	$Amy^{1,2}$	0.042± 0.018
$Amy^{2,3}/Amy^{1,3}$	3	$Amy^{1,6}$	0.008± 0.008
$Amy^{1,3}/Amy^{1}$	3	$Amy^{2,3}$	0.085± 0.026
$Amy^{1,2}/Amy^{1,3}$	2		
$Amy^{1,6}/Amy^{1}$	1		
Total	59		

The flies typed for the Wisconsin population were not the samples taken directly from nature, but since the number of generations kept in the laboratory cultures was small, they could be considered as samples that came directly from the natural population.

From Table IV, it can be seen that Amy^{1} is again the most common with $Amy^{1,3}$ being present in a fairly high frequency. Deviation from the Hardy-Weinberg distribution was examined by grouping $Amy^{1,3}$, $Amy^{1,2}$, $Amy^{2,3}$ and $Amy^{1,6}$ as one class; expected and observed frequency agreed quite well ($X^2=0.45$, d.f.=1, $p<0.05$). Not much information, however, can be

obtained from this kind of analysis for the present data.

GENERAL DISCUSSION

Certain similarities in the frequencies of amylase alleles may be seen among the populations in this survey with particular emphasis being placed on the Texas and Wisconsin populations. In all cases *Amy*^{1} is, by a considerable margin, the most frequent with the *Amy*^{1,3} gene being the second most frequent in both the Texas and Wisconsin populations. These two genes made up 95% of the frequency in the Texas population and 87% of the frequency of the Wisconsin sample. The same amylase alleles were found in both the Texas and Wisconsin populations. The *Amy*^{1,2} was observed in a low frequency in both populations and the *Amy*^{1,6} was quite rare in both populations. Perhaps the largest difference between these two populations would be the higher frequency of *Amy*^{2,3} in the Wisconsin population (8%) versus the Texas population (1%).

Because of the reasons mentioned at the beginning of this chapter, the frequencies of amylase alleles found for the Odate population in this survey may not reflect the exact constitutions in nature. In any event, the observed frequencies in the Odate population appeared to be somehow similar to those found in the Wisconsin and Texas populations.

In summary, it may be concluded that the amylase isozymes do exist in a polymorphic state in many natural populations of *Drosophila melanogaster*; the most common allele is *Amy*^{1} with the approximate frequency of 80%, and

the next most frequent allele is $\underline{Amy}^{1,3}$ with the frequency of about 10%. Because of these higher frequencies, most of the remainder of this study will be devoted to the study of Amy^{1} and $Amy^{1,3}$ alleles in the Texas and Wisconsin populations.

CHAPTER III

VIABILITY EXPERIMENT

The viability experiment consisted of two separate sets of experiments. The first set of experiments to be discussed involved the mating of Cy/$Amy^{1(or\ 1,3)}$ x Cy/$Amy^{1(or\ 1,3)}$. The amylase type of the Cy chromosome is fixed for Amy^1. The three mating types in regard to Amylase types are: $^1/1$ x $^1/1$, $^1/1$ x $^1/1,3$, and $^1/1,3$ x $^1/1,3$ with the genotypes of the non-Curly progeny being respectively $^1/1$, $^1/1,3$, $^{1,3}/1,3$.

The second set of experiments which will be more fully discussed later in this chapter involved the cross of one cn,bw, Amy^1/+, +, $Amy^{1(or\ 1,3)}$ male to two cn,bw, Amy^1/cn,bw, Amy^1 females. The progeny were classified into cn,bw males, cn bw females, + males, and + females. All of the chromosomes from the cn bw stock were fixed for Amy^1.

Cy/Amy x Cy/Amy EXPERIMENT: METHOD

From each of the backcross lines, which are shown in Figure 1, one or two cn bw/+ males were crossed with several Cy/cn bw females. The + always refers to one of the chromosomes taken from natural populations. The Cy chromosome also carries the mutant cn^2, which is an allele of cn, and this permits one to distinguish Cy/cn bw from Cy/+ in the offspring from the above mating. In this manner, forty different lines of Cy/+ were established with ten lines each of Texas Amy^1, Texas $Amy^{1,3}$, Wisconsin Amy^1, and

Wisconsin $\underline{Amy}^{1,3}$. After establishing these lines, all of the various combinations both within and between populations were made in the following mating which is diagrammed below.

$$\begin{array}{ccc} 2\,♀ & & 2\,♂ \\ Cy/+ & + & Cy/+ \end{array}$$

---

dies	Cy/+		+/+
	2 Cy	:	1 non-Cy

These matings also included reciprocal matings. By excluding those crosses which gave +/+ progeny that were homozygous for the entire second chromosome (Cy/+ x Cy/+ matings within the same line), there were a total of 1,560 crosses made.

Two pairs of flies, which were less than 1.5 days old, were placed into a vial for four days, then they were transferred to a second vial for four more days, and finally transferred to a third vial for an additional four days before being discarded. This procedure minimized crowding conditions, but it still permitted a relatively large number of offspring (80 or 90 per mating) to be counted. The emerging progeny were classified according to sex and wing type into the four phenotypic classes of $\underline{Cy}$ female, $\underline{Cy}$ male, + female, and + male. From each culture k values were calculated with k being defined thusly: $k = \frac{\text{number of +/+ progeny}}{\text{Total number of progeny}}$. These k values were computed for each sex separately with ko referring to the k value for the male progeny and $k♀$ for the

female progeny. Since a considerable number of the k values were less than 0.3, the arcsin transformation was employed to transform the k values (Snedecor and Cochran, 1967).

GENERAL COMMENTS ON VIABILITY STUDIES

The use of the Cy/+ ♀ x Cy/+ ♂ mating has been quite common in viability studies concerning the second chromosome. In these studies the viability of the +/+ fly was measured by the frequency of the surviving +/+ flies relative to the standard Cy/+, assuming that the Cy chromosome is very nearly dominant in viability over the + chromosomes. Through the experience of many workers this assumption has been known to be true.

Another technique, which utilizes the mating of +/Cy, L ♀ x +/Pm ♂, was employed by Wallace (1956) and is known as the Cy-L / Pm technique. This technique permits one to have a standard genotypic progeny (Cy-L / Pm) which is independent of the chromosomes being tested. While this technique has the advantage of the standard being independent of the tested chromosomes, it has the practical disadvantage of giving four phenotypic classes of progeny flies with smaller numbers of flies in each class. In addition, the phenotype of the Pm variagation becomes somewhat obscured and may cause difficulty in distinguishing it from the +/+ flies. For the convenience in handling a large number of matings and for obtaining a large number of progeny flies, the +/Cy ♀ x +/Cy ♂ matings were employed in this study. At any rate,

the second part of this chapter does utilize progeny of a standard (cn bw/cn bw) genotype.

Comparison of ko♂ with k♀+

In most viability studies concerning the second chromosome, the segregation frequency, or viability, has been estimated without separating the progeny flies into the two sexes on the assumption of the same viability between the sexes. In the present study the two phenotypic classes of Cy/+ and +/+ were subdivided into sexes to see if viability differences are the same in the two sexes. The results of the k♀+ - ko♂ comparison is illustrated by Table VA. In this table and other similar tables in this chapter the amylase genotype refers to the +/+ flies from the Cy/+ x Cy/+ mating. The $\overline{ko}$♂ and $\overline{k}$♀+ are the average k values for each sex and N stands for the number of crosses made for each respective class.

TABLE VA

ko - k ♀+ COMPARISON

Amy genotype	$\overline{ko}$♂	N	$\overline{k}$♀+	N
Amy^1/Amy^1	35.81	380	37.21	380
$Amy^1/Amy^{1,3}$	37.06	800	37.89	800
$Amy^{1,3}/Amy^{1,3}$	38.12	380	37.95	380
Total	37.01	1560	37.74	1560

It can be seen from this table that the $\overline{ko}$ shows a much greater increase in $\overline{k}$ in proceeding from the Amy^1/Amy^1

genotype to the $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ genotype, although the same general tendency is seen in both sexes. The overall average of k is higher for the females than the males. The analysis of variance for this data is shown by Table VB.

TABLE VB

ANALYSIS OF VARIANCE FOR THE $k♀ - ko♂$ COMPARISON

SOURCE	S.S.	d.f.	M.S.	F
Between $k♀$ and ko	410.16	1	410.16	6.31**
Between amylase genotypes	923.27	2	461.63	7.10***
Interaction	243.34	2	121.67	1.87
Between genotypes within subclass	101,077.51	1554	65.04	1.66***
Between reciprocal matings	61,238.53	1560	39.26	

**: $p < 0.01$ ***: $p < 0.005$

From Table VB, it may be observed that the differences between $k♀$ and $ko♂$ was highly significant and the differences among the three amylase genotypes was highly highly significant. The variance among genotypes within subclass was highly highly significant when compared with that between reciprocal matings and was consequently used as the error term for the F tests.

Comparison of lethals versus non-lethals

Hiraizumi and Crow (1960) observed about 29% (212/735) of the second chromosomes contained recessive lethals and

semilethals from a natural population of Drosophila melanogaster trapped in Madison, Wisconsin. Wallace (1968) reported a frequency of about one lethal out of every five or six second chromosomes; another earlier survey of a particular tropical population in Bogota, Columbia by Wallace, Zouros, and Krembas (1966) revealed a frequency of lethals and semilethals of about 55%. There have been many other observations made but there is a general agreement that recessive lethals are fairly common on second chromosomes taken from natural populations.

Some studies have shown the viability of flies containing a recessive lethal in the heterozygous condition to be depressed when compared with non-lethals (Stern C., et.al. 1952; Hiraizumi and Crow, 1960); however, there have also been examples of specific cases of a heterotic effect of a lethal in heterozygotes (Mukai and Burdick, 1959). The purpose of this study is not to study the effects of lethals on viability; nevertheless, it should be acknowledged that a great deal of research has been done which indicates an significant effect of lethals on viability. In the present study the total matings were divided into a group involving lethals and a group without lethals with the k values for the three amylase genotypes being compared for each group.

There was a total of five lethals in the forty chromosome lines. Three of these were in the Amy^{1} lines (W(1)-2 lethals; T(1)-1 lethal) and two were inthe $Amy^{1,3}$ lines (T(1,3)-

2 lethals; W(1,3) - no lethals). Since there was a significant difference between $k\sigma^{\nearrow}$ and $k\,♀$, and to make the analysis simple, the comparison between lethals and non-lethals was made separately for each sex. This lethal versus non-lethal comparison for $k\sigma^{\nearrow}$ and the analysis of variance is shown in Table VIA. The same type of analysis for $k\,♀$ is shown by Table VIB.

It may be seen from these tables that the lethals do show a significantly reduced k value ($p < 0.05$) from the non-lethals in the male progeny only. The same general trend is observed in the females but the difference is not significant.

TABLE VIA

COMPARISON AND ANALYSIS OF VARIANCE FOR

LETHAL VERSUS NON-LETHAL FOR ko

	LETHAL		NON-LETHAL	
Amy Genotype	$\bar{k}\sigma^{\nearrow}$	N	$\bar{k}\sigma^{\nearrow}$	N
Amy^{1}/Amy^{1}	34.96	108	36.15	272
$Amy^{1}/Amy^{1,3}$	35.85	188	37.43	612
$Amy^{1,3}/Amy^{1,3}$	39.00	74	37.91	306
TOTAL	36.22	370	37.26	1,190

ANALYSIS OF VARIANCE

SOURCE OF VARIANCE	S.S.	d.f.	M.S.	F
Between lethal and non-lethal	229.03	1	229.03	3.81*
Between amylase genotypes	944.12	2	472.06	7.85***
Interaction	308.97	2	154.49	2.57
Between genotypes within subclass	46,514.62	774	60.33	1.63***
Between reciprocal matings	28,710.84	780	36.81	

*: $p < 0.05$ ***: $p < 0.005$

TABLE VIB

COMPARISON AND ANALYSIS OF VARIANCE FOR LETHAL VERSUS NON-LETHAL FOR k_{φ}^{φ}

	LETHAL		NON-LETHAL	
Genotype	$\bar{k}_{\varphi}^{\varphi}$	N	$\bar{k}_{\varphi}^{\varphi}$	N
Amy^1/Amy^1	36.93	108	37.32	272
$Amy^1/Amy^{1,3}$	37.35	188	38.06	612
$Amy^{1,3}/Amy^{1,3}$	38.88	74	37.73	306
TOTAL	37.53	370	37.81	1,190

ANALYSIS OF VARIANCE

SOURCE OF VARIANCE				
Between lethal and non-lethal	34.33	1	34.33	0.49
Between Amylase genotype	157.77	2	78.89	1.13
Interaction	147.68	2	73.84	1.06
Between Genotypes within Subclass	54,152.72	774	69.96	1.68***
Between reciprocal matings	32,461.64	780	41.62	

***: $p < 0.005$

Therefore there was either no association between the lethal heterozygous effect and the viability of a specific amylase allele, or the lethal heterozygous effect was homogeneous among the amylase types. In neither the ko nor k♀+ analysis was there any significant interaction between lethals and amylase genotype.

Comparisons of populations

In the Cy/+ x Cy/+ matings, the origin of the amylase lines was from either a natural population in Texas or one in Wisconsin. From these populations three mating types were made within each amylase combinations; those involving only the Wisconsin population (W/W), those involving only the Texas population (T/T), and those involving interpopulational

matings between Texas and Wisconsin (T/W).

The analysis for the populations was subdivided into $k\sigma^{\nearrow}$ - $k\varphi$ and lethal versus non-lethal classes. The results are shown on Tables VIIA through VIID.

The following conclusions may be drawn from this analysis.

1. The three amylase genotypes showed in both non-lethal and lethal groups, significant difference in the ko, but not in the $k\varphi$.
2. There are no significant difference between the three population groups.
3. Since the three population groups showed a relatively consistent relation of k among the three amylase genotypes, there was no significant interaction between the amylase types and the populations.

TABLE VIIA

COMPARISON OF POPULATIONS WITH AMYLASE TYPES

IN kσ♂ NON-LETHAL GROUPS

	AMYLASE GENOTYPES					
	Amy^1/Amy^1		$Amy^1/Amy^{1,3}$		$Amy^{1,3}/Amy^{1,3}$	
Population	$\bar{k}$	N	$\bar{k}$	N	$\bar{k}$	N
W/W	34.74	56	37.72	160	37.99	90
T/T	36.49	72	37.14	144	37.08	56
W/T	36.52	144	37.42	308	38.16	160

ANALYSIS OF VARIANCE

SOURCE	S.S.	d.f.	M.S.	F
Between amylase genotypes	479.71	2	239.86	4.03*~**
Between populations	31.65	2	15.82	0.27
Interaction	183.11	4	45.78	0.77
Between genotypes within subclass	34,880.93	586	59.52	1.78***
Between reciprocal matings	19,940.35	595	33.51	

*~**: $p < 0.02$ ***: $p < 0.005$

TABLE VIIB

COMPARISON OF POPULATIONS WITH AMYLASE TYPES

IN k^{φ}_{+} NON-LETHAL GROUPS

	AMYLASE GENOTYPES					
	Amy^1/Amy^1		$Amy^1/Amy^{1,3}$		$Amy^{1,3}/Amy^{1,3}$	
Population	$\bar{k}$	N	$\bar{k}$	N	$\bar{k}$	N
W/W	37.95	56	37.87	160	38.67	90
T/T	37.48	72	37.91	144	37.15	56
W/T	36.99	144	38.23	308	37.39	160

ANALYSIS OF VARIANCE

SOURCE	S.S.	d.f.	M.S.	F
Between amylase genotypes	101.21	2	50.61	0.68
Between populations	36.73	2	18.36	0.25
Interaction	138.72	4	34.68	0.47
Between genotypes within subclass	43,473.15	586	74.12	1.87***
Between reciprocal matings	23,551.99	595	39.58	

***: $P < 0.005$

TABLE VIIC

COMPARISON OF POPULATIONS WITH AMYLASE TYPES

IN kσ♂ LETHAL GROUPS

	AMYLASE GENOTYPES					
	Amy^1/Amy^1		$Amy^1/Amy^{1,3}$		$Amy^{1,3}/Amy^{1,3}$	
Population	$\bar{k}$	N	$\bar{k}$	N	$\bar{k}$	N
W/W	34.55	34	37.45	40	-	-
T/T	37.24	18	35.46	56	38.80	34
W/T	34.47	56	35.40	92	39.16	40

ANALYSIS OF VARIANCE

SOURCE	S.S.	d.f.	M.S.	F
Between amylase genotypes	774.87	2	387.44	6.05***
Between populations	62.29	2	31.15	0.49
Interaction	182.84	3	60.95	0.95
Between genotypes within subclass	11,327.24	177	64.00	1.26*
Between reciprocal matings	9,420.50	185	50.92	

*: $p < 0.05$ ***: $p < 0.005$

TABLE VIID

COMPARISON OF POPULATIONS WITH AMYLASE TYPES IN $k\frac{♀}{+}$ LETHAL GROUPS

	AMYLASE GENOTYPES					
	Amy^1/Amy^1		$Amy^1/Amy^{1,3}$		$Amy^{1,3}/Amy^{1,3}$	
Population	$\bar{k}$	N	$\bar{k}$	N	$\bar{k}$	N
W/W	35.93	34	38.93	40	-	-
T/T	39.50	18	36.75	56	38.81	34
W/T	36.72	56	37.02	92	38.94	40

ANALYSIS OF VARIANCE

SOURCE	S.S.	d.f.	M.S.	F
Between amylase genotypes	185.39	2	92.69	1.45
Between populations	23.42	2	11.71	0.18
Interaction	258.14	3	86.05	1.35
Between genotypes within subclass	10,640.53	177	60.12	1.25
Between reciprocal matings	8,913.31	185	48.12	

*: $p < 0.05$

Comparison of amylase genotypes

This analysis involved the following two comparisons of Amy^{1}/Amy^{1} versus $Amy^{1}/Amy^{1,3}$, and $Amy^{1}/Amy^{1,3}$ versus $Amy^{1,3}/Amy^{1,3}$. In almost all of the previous analysis the greatest difference in k values was between the two amylase homozygotes with the amylase heterozygote being somewhere intermediate. Since the differences between amylase types in the $k♀$ was not significant, this analysis was done only for the ko progeny. The lethal and non-lethal groups were analyzed separately, but the three population groups were pooled as there was neither a significant difference between populations nor was the interaction significant. The results of the $ko♂$ non-lethals and $ko♂$ lethals are shown by Tables VIIIA through VIIID.

TABLE VIIIA

ANALYSIS OF VARIANCE BETWEEN AMYLASE GENOTYPES

FOR kσ♂ NON-LETHALS

SOURCE	S.S.	d.f.	M.S.	F
Between amylase genotypes **(1/1 versus 1/1,3)	311.55	1	311.55	4.78*
Between genotypes within subclass	28,658.02	440	65.13	1.92***
Between reciprocal matings	15,001.82	442	33.94	

*: $p < 0.05$ ***: $p < 0.005$

** The designation of the amylase genotypes is shortened by dropping the Amy abbreviation; for example Amy^1/Amy^1 is shortened to 1/1.

TABLE VIIIB

ANALYSIS OF VARIANCE BETWEEN AMYLASE GENOTYPES

FOR kσ♂ NON-LETHALS

SOURCE	S.S.	d.f.	M.S.	F
Between amylase genotypes (1/1,3 versus ^{1,3}/1,3)	47.01	1	47.01	1.01
Between genotypes within subclass	21,298.84	457	46.61	1.40***
Between reciprocal matings	15,266.12	459	33.26	

***: $p < 0.005$

TABLE VIIIC

ANALYSIS OF VARIANCE BETWEEN AMYLASE GENOTYPES

FOR k♂ LETHALS

SOURCE	S.S.	d.f.	M.S.	F
Between amylase genotypes (1/1 versus 1/1,3)	55.34	1	55.34	0.85
Between genotypes within subclass	9,536.95	146	65.32	1.13
Between reciprocal matings	8,578.96	148	57.89	

TABLE VIIID

ANALYSIS OF VARIANCE BETWEEN AMYLASE GENOTYPES

FOR k♂ LETHALS

SOURCE	S.S.	d.f.	M.S.	F
Between amylase genotypes (1/1,3 versus 1,3/1,3)	524.33	1	524.33	8.99***
Between genotypes within subclass	7,524.70	129	58.33	1.72**
Between reciprocal matings	4,439.05	131	33.89	

**: $p < 0.01$ ***: $p < 0.005$

The differences in k_{o}^{σ} between the three amylase genotypes are not exactly the same between lethals and non-lethals. The k value of the amylase heterozygote lies closer to the lowest k value of the 1/1 amylase type for the lethals, but for the non-lethals it lies closer to the highest value of the 1,3/1,3 amylase type. Nevertheless, the k value for the 1/1,3 heterozygote lies somewhere between the two homozygotes, and this relation was consistent in the three population groups. There was no indication of any heterozygous advantage in k.

SEX RATIO

In the previous analyses k_{o}^{σ} was significantly different among the three amylase genotypes. The k_{+}^{φ} showed the same tendency as k_{o}^{σ}, but the difference was much smaller and was not statistically significant. The most likely hypothesis of these results would be that the male responds with greater sensitivity to viability changes than does the female. This could be tested by comparing the sex ratios (proportion of males) separately for the two segregating genotypes. If this interpretation is valid, one would expect to find the lowest sex ratio for the 1/1 homozygote, highest for the 1,3/1,3 homozygote, and intermediate for the 1/1,3 heterozygote. In addition, no such sex ratio change should be expected in the Cy/+ class between the three amylase matings types provided the viability of the Cy flies are the same regardless of the homologue of the Cy chromosome. The SR

values (sex ratio) were computed for each amylase mating type separately for each of the two segregating genotypes and were subjected to statistical analysis. The average SR and analysis of variance for the Cy/+ non-lethal progeny is given by Table IX.

TABLE IX

AVERAGE SR AND ANALYSIS OF VARIANCE

FOR THE Cy/+ PROGENY

	AMYLASE MATING TYPES					
	1/Cy x 1/Cy		1/Cy x ^{1,3}/Cy		^{1,3}/Cy x ^{1,3}/Cy	
POPULATIONS	SR	N	SR	N	SR	N
W x W	0.564	56	0.510	160	0.521	90
T x T	0.525	72	0.531	144	0.494	56
W x T	0.513	144	0.516	308	0.502	160
TOTAL	0.527	272	0.518	612	0.506	306

ANALYSIS OF VARIANCE

SOURCE	S.S	d.f.	M.S.	F
Between amylase mating types	0.0608	2	0.0304	3.85*⌣**
Between populations	0.0365	2	0.0183	2.32
Interaction	0.0384	4	0.0096	1.22
Between chromosomal combinations	4.6501	586	0.0079	1.03
Between reciprocal crosses	4.5776	595	0.0077	

*⌣**: $p < 0.02$

The results of the variance analysis for the +/+ progeny did not show any significant difference among the three amylase genotypes. There was a slightly higher SR value among the +/+ progeny of the $Cy/Amy^{1,3}$ x $Cy/Amy^{1,3}$ mating type (0.510) versus the SR among the progeny of the Cy/Amy^{1} x Cy/Amy^{1} mating type (0.505) but the difference was not statistically significant. In contrast to this, the SR among the Cy progeny (shown by Table IX) is significantly higher for the 1 x 1 mating type versus the 1,3 x 1,3 mating type. These results rule out the hypothesis mentioned above.

The possibility of non-random assortment between the second and sex chromosomes as was reported by Sakai and Hiraizumi (1969) appears unlikely in this instance because the reciprocal amylase mating types ($\underline{Amy}^{1,3}/\underline{Cy}+^{\varphi}$ x $\underline{Amy}^{1}/\underline{Cyo}^{\sigma}$ versus $\underline{Amy}^{1}/\underline{Cy}+^{\varphi}$ x $\underline{Amy}^{1,3}/\underline{Cyo}^{\sigma}$) did not show a significant difference in the SR among the Cy progeny. One possible explanation may be that the dominancy in viability of the Cy chromosome over the + chromosome is not complete, but is somewhat dependent upon its homologous + chromosome. In any event it is still safe to conclude that the viability of $\underline{Amy}^{1}/\underline{Amy}^{1}$ is the lowest, $\underline{Amy}^{1}/\underline{Amy}^{1,3}$ is intermediate, and $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ is the highest.

SUMMARY OF THE Cy/+ x Cy/+ VIABILITY STUDY

Very briefly the following conclusions from this part of the viability study may be drawn.

1. There highly are significant differences between $k\sigma^{\nearrow}$ and $k\varphi$ although the same general tendency in regard to Amylase type was observed in both sexes.

2. There were significant differences in $k\sigma^{\nearrow}$ between those crosses involving a recessive lethal and those not involving recessive lethals, but no significant difference was found in $k\varphi$.

3. There was no significant difference in k between the population groups.

4. There was a significant difference in $k\sigma^{\nearrow}$ among the three amylase genotypes in both lethal and non-lethal groups. In both lethals and non-lethals, $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ had the highest $k\sigma^{\nearrow}$ value with $\underline{Amy}^{1}/\underline{Amy}^{1}$ always having the lowest value. The same relationship was found to be consistent between $k\sigma^{\nearrow}$ amylase genotypes in the three population groups. These same general tendencies were observed for $k\varphi$, but the differences were much smaller, less consistent, and were therefore not statistically significant.

Backcross Experiment: Methods

This second set of viability experiments was carried out by crossing three males from each of the backcross lines to three cn bw virgin females. The parents were less than five days old (usually less than three days old) when mated.

After the parental flies had been in the culture vial for twenty-four hours, they were transferred to a fresh culture for three days before being discarded. The purpose of making the pre-mating was to insure that most of the females had been fertilized and would quickly begin depositing eggs in the fresh vial.

The F_1 progeny were counted and classified into the four phenotypic types of cn bw females, cn bw males, + females, and + males. These matings generally produced 90-100 offspring per vial. The k value is defined as the number of + progeny divided by the total number of offspring. The expected k value for the backcross experiment was 0.5 and since these values were nearly always between 0.4 and 0.6, the arcsin transformation was not applied.

The backcross experiment would detect any chromosomes carrying Segregation Distorter (SD); whereas it would be undetected in the +/Cy x +/Cy matings since the Cy inversion suppresses the action of SD (Sandler, Hiraizumi, and Sandler, 1959). Of the chromosomes tested, none appeared to carry SD. As was mentioned at the beginning of this chapter, the viability of the standard genotype for the backcross experiment (cn bw/cn bw progeny) is independent of the chromosome being tested which is not true for the Cy/+ standard.

In respect to the amylase genotypes, there were no $Amy^{1,3}/Amy^{1,3}$ flies in the backcross experiment since cn bw

is fixed for Amy^1. This study compares differences between only two amylase genotypes in contrast to the Cy/+ x Cy/+ study which had compared three genotypes.

COMPARISON OF $\bar{k}$ OF AMY^1 AND $AMY^{1,3}$

Because the most common amylase alleles are $\underline{Amy}^1$ and $\underline{Amy}^{1,3}$, most of the backcross experiment was concerned with these two alleles. A total of 41 $\underline{Amy}^1$ and 44 $\underline{Amy}^{1,3}$ chromosome lines were examined with an average of 12.35 replications per line. A preliminary analysis was made with both sexes combined. The average k values and analysis of variance are given by Table X.

TABLE X

AVERAGE k VALUES AND ANALYSIS OF VARIANCE FOR AMY^1 AND $AMY^{1,3}$ BACKCROSS EXPERIMENT

POPULATION	N	$\bar{k}(AMY^1)$	N	$\bar{k}(AMY^{1,3})$	N	(TOTAL) $\bar{k}$
TEXAS	205	0.5025	255	0.5062	460	0.5046
WISCONSIN	303	0.5116	290	0.5212	590	0.5163
TOTAL	505	0.5079	545	0.5142	1050	0.5112

ANALYSIS OF VARIANCE

SOURCE	S.S.	d.f.	M.S.	F
Amylase genotypes	0.0129	1	0.0129	0.61
Populations	0.0485	1	0.0485	2.29
Interaction	0.0023	1	0.0023	0.11
Between chromosome lines	1.7176	81	0.0212	6.06***
Within chromosome lines	3.3415	965	0.0035	

***: $p < 0.005$

Although the $\bar{k}$ value for $\underline{Amy}^{1,3}$ is higher than $\underline{Amy}^1$ in both populations, this difference is not significant. The Wisconsin population has a consistently higher $\bar{k}$ value than the Texas population, but this was also not significant. The interaction between amylase genotypes with populations is very small. As was expected, the variance between chromosome lines was highly highly significant and was used

as the error term in the analysis.

COMPARISON OF $k\sigma^{\male}$ WITH $k\overset{\female}{+}$

This is the same type of comparison made in the previous Cy viability experiment. The average k values and analysis of variance are shown by Table XI. The two populations were pooled together in this analysis.

From Table XI, it may be observed that there is a highly highly significant difference between $k\sigma^{\male}$ and $k\overset{\female}{+}$ with $\overline{k\sigma^{\male}}$ being higher than the $\overline{k\overset{\female}{+}}$. The variation between the amylase types and the interaction term was not significant.

TABLE XI

$\bar{k}o^{\nearrow}$ - $\bar{k}^{\varphi}$ COMPARISON AND ANALYSIS OF VARIANCE

	N	$\bar{k}(AMY^1)$	N	$\bar{k}(AMY^{1,3})$	TOTAL $\bar{k}$
$ko^{\nearrow}$	505	0.5117	545	0.5244	0.5183
k^{φ}	505	0.5018	545	0.5049	0.5034

ANALYSIS OF VARIANCE

SOURCE	S.S.	d.f.	M.S.	F
$ko^{\nearrow}$ - k^{φ}	0.1161	1	0.1161	9.76***
Amylase genotypes	0.0327	1	0.0327	2.75
Interaction	0.0121	1	0.0121	1.02
Between chromosome lines	1.9716	166	0.0119	1.83***
Within chromosome lines	12.4846	1930	0.0065	

***: $p < 0.005$

COMPARISON OF POPULATIONS WITH AMYLASE GENOTYPES

Because of the large differences between $ko^{\nearrow}$ and $\bar{k}^{\varphi}$, these two were tested separately. The averages for $ko^{\nearrow}$ and $ko^{\nearrow}$ for each population are given by Table XIIA, and the analyses of variance for $ko^{\nearrow}$ and k^{φ} are shown by Table XIIB.

Based upon these results the following concousions may be drawn:

TABLE XIIA

AVERAGES OF k_{σ}^{σ} AND k_{+}^{φ} IN EACH POPULATION

		AMYLASE TYPES				
	POPULATIONS	N	$\bar{k}(AMY^{1})$	N	$\bar{k}(AMY^{1,3})$	$\bar{k}$(TOTAL)
k_{σ}^{σ}	TEXAS	205	0.5090	255	0.5181	0.5140
	WISCONSIN	300	0.5135	290	0.5300	0.5216
	TOTAL k_{σ}^{σ}	505	0.5117	545	0.5244	
	TEXAS	205	0.4921	255	0.4964	0.4945
k_{+}^{φ}	WISCONSIN	300	0.5084	290	0.5124	0.5104
	TOTAL k_{+}^{φ}	505	0.5018	545	0.5049	

TABLE XIIB

ANALYSIS OF VARIANCE FOR k_{σ}^{σ} AND k_{+}^{φ}

ANALYSIS OF VARIANCE FOR k_{σ}^{σ}

SOURCE	S.S.	d.f.	M.S.	F
Amylase genotype	0.0460	1	0.0460	4.42*
Population	0.0183	1	0.0183	1.76
Interaction	0.0035	1	0.0035	0.34
Between chromosome lines	0.8406	81	0.0104	1.73**
Within chromosome lines	5.8071	965	0.0060	

*: $p < 0.05$ **: $p < 0.01$

ANALYSIS OF VARIANCE FOR $k_{♀}$

SOURCE	S.S.	d.f.	M.S.	F
Amylase genotype	0.0044	1	0.0044	0.34
Population	0.0688	1	0.0688	5.14*
Interaction	0.0001	1	0.0001	0.01
Between chromosome lines	1.0529	81	0.0130	1.88**
Within chromosome lines	6.6611	965	0.0069	

*: $p < 0.05$ **: $p < 0.01$

1. The $\underline{Amy}^{1}/\underline{Amy}^{1,3}$ genotype displays a significantly higher $k_{♂}$ value than the $\underline{Amy}^{1}/\underline{Amy}^{1}$ genotype, although the difference was not highly significant.

The same tendency is observed in the $k_{♀}$, but the difference is not significant. This was consistent in both the Texas and Wisconsin populations.

2. The Wisconsin population has a significantly higher k value than the Texas population. The same tendency is also seen for the $k_{♂}$.

COMPARISON OF LETHAL VERSUS NON-LETHALS

There were thirteen recessive lethal-bearing chromosomes among the eighty-five tested chromosomes which were distributed more or less at random in regard to the amylase genotypes with seven in the $\underline{Amy}^{1}$ lines and six in the $\underline{Amy}^{1,3}$ lines. The lethal and non-lethal crosses were compared for

the ko♂ and k♀. The averages for these two classes are shown in Table XIII.

TABLE XIII

COMPARISON OF LETHALS WITH NON-LETHAL CHROMOSOMES

			AMYLASE GENOTYPES			
			AMY^1/AMY^1		$AMY^1/AMY^{1,3}$	
		POPULATIONS	N	$\bar{k}$	N	$\bar{k}$
ko♂	non-lethal	TEXAS	195	0.5104	176	0.5146
		WISCONSIN	230	0.5139	260	0.5280
	lethal	TEXAS	10	0.4820	79	0.5258
		WISCONSIN	70	0.5122	30	0.5473
k♀	non-lethal	TEXAS	195	0.4928	176	0.5002
		WISCONSIN	230	0.5120	260	0.5115
	lethal	TEXAS	10	0.4800	79	0.4879
		WISCONSIN	70	0.4964	30	0.5202

The average ko♂ for lethal and non-lethal class was 0.5219 and 0.5174 respectively, and the average k♀ for the respective class was 0.4958 and 0.5051. In neither case was the difference significant. Thus there was no evidence for lethal heterozygous effects on viability, but since the number of lethal chromosomes was small, this cannot be considered as conclusive.

THE $AMY^{1}/AMY^{1,2}$ GENOTYPE

Since the number of chromosome lines containing $\underline{Amy}^{1,2}$ was small, the number of crosses involving this chromosome was also rather small. The averages were computed for only non-lethals and involved four chromosome lines from each population. The average ko values of $\underline{Amy}^{1,2}$ was 0.5245 for Texas and 0.5256 for the Wisconsin population, while the average $k_{♀}$ was 0.5008 and 0.5089 for the respective populations. Thus the viability of $\underline{Amy}^{1,2}$ appear similar to $\underline{Amy}^{1,3}$, but the number of independent chromosomes tested was too small to obtain any conclusions.

SUMMARY OF THE BACKCROSS MATING EXPERIMENT

1. The $\underline{Amy}^{1,3}/\underline{cn}\ \underline{bw}$ genotype showed, on the average, a higher ko than that of $\underline{Amy}^{1}/\underline{cn}\ \underline{bw}$. The same relation was also found in $k_{♀}$, but the difference was much smaller and was not satistically significant. The $\underline{Amy}^{1,2}/\underline{cn}\ \underline{bw}$ genotype exhibited a similar viability as $\underline{Amy}^{1,3}/\underline{cn}\ \underline{bw}$, but the number of chromosome lines tested was small and the results were not conclusive.
2. The $ko_{♂}$ was, for both of the amylase genotypes higher than $k_{♀}$.
3. There was an indication suggesting some difference in k values between the two populations with the Wisconsin population having a higher k.
4. There was no evidence for the lethal heterozygous effect on viability, but the number of lethal chromosomes tested was too small to draw any conclusions.

CHAPTER IV

FERTILITY

During the course of the +/Cy x +/Cy viability experiment, a very interesting phenomenon was found; i.e., $\underline{Amy}^{1,3}/\underline{Cy}$ female showed, as compared with $\underline{Amy}^{1}/\underline{Cy}$ female, a consistently higher fertility (as noted before, overcrowding conditions were avoided and practically all of the fertilized viable zygotes produced during the 12 days period were sampled). The average numbers of progenies per two pairs (lethals were excluded) are summarized in Table XIV.

TABLE XIV

AVERAGE NUMBER OF PROGENY PRODUCED IN Cy/+ x Cy/+ NON-LETHAL CROSSES FROM THE VIABILITY STUDY

CROSS: ♀ Cy/+ x ♂ Cy/+

♀ \ ♂	W(1)	T(1)	W(1,3)	T(1,3)	Total
W(1)	N= 56	N= 72	N= 80	N= 64	N=272
	$\bar{x}$= 75.5	$\bar{x}$= 78.9	$\bar{x}$= 71.9	$\bar{x}$= 85.9	$\bar{x}$= 77.8
T(1)	N= 72	N= 72	N= 90	N= 72	N=306
	$\bar{x}$= 85.1	$\bar{x}$= 68.8	$\bar{x}$= 90.4	$\bar{x}$= 80.1	$\bar{x}$= 81.6
W(1,3)	N= 80	N= 90	N= 90	N= 80	N=340
	$\bar{x}$= 90.7	$\bar{x}$= 90.8	$\bar{x}$= 85.3	$\bar{x}$= 96.7	$\bar{x}$= 90.7
T(1,3)	N= 64	N= 72	N= 80	N= 56	N=272
	$\bar{x}$=103.8	$\bar{x}$= 93.9	$\bar{x}$=103.2	$\bar{x}$= 96.3	$\bar{x}$= 99.5
TOTAL	N=272	N=306	N=340	N=272	N=1190
	$\bar{x}$= 89.2	$\bar{x}$= 83.6	$\bar{x}$= 87.7	$\bar{x}$= 89.7	$\bar{x}$= 87.4

In this table, Texas and Wisconsin populations are abbreviated with T and W respectively and the amylase type of the + chromosome is abbreviated by simply writing the band number (1 or 1,3). The number of the mating is designated by N and the average number of progeny by $\bar{X}$. The higher fertility of the 1,3/Cy female is clearly seen from this table, with no difference among male genotypes. This mating, +/Cy x +/Cy, was designed for the viability study and was therefore not a suitable mating for the fertility study, but the results given in Table XIV strongly suggested a fairly large fertility differences among amylase genotypes. Accordingly, a more refined experiment was designed to study the fertility differences among amylase genotypes.

FERTILITY FOR AMY^1 AND $AMY^{1,3}$: MATERIALS AND METHODS

From the Cy/+ x Cy/+ matings, +/+ females of the appropriate combinations were collected. The three amylase genotypes for these females are 1/1, 1/1,3, and $^{1,3}/1,3$ with the origin of the chromosomes in respect to populations being T/T, W/W, and T/W. This gives a total of nine subgroups. A total of fourteen Amy^1 chromosomes and fifteen $Amy^{1,3}$ chromosomes were examined. No chromosomes carrying recessive lethals were included in this study. A total of 158 chromosome combinations were made (completely homozygous chromosome combinations were excluded) with a total of 1484 crosses. There were approximately ten replications for each chromosome combination.

Each +/+ female was one or two days old when crossed with four cn bw males which were more than one and less than five days old. These flies were transferred every fourth day for a total of four times to fresh culture vials and were then discarded at the end of the fourth day after the final transfer. If the number of surviving males was two or less, fresh cn bw males were added to the culture vial. This mating scheme avoided overcrowded conditions in the culture vials. The differential viability could also influence the number of progeny, but such as effect would be rather small.

Although the present experiment does not provide the total number of progeny per female, it does test the fertility during the most important period of the early stages of adult life. The average lifetime of the adult fly in nature is unknown, but it is almost certainly less than twenty days. Frydenberg (1961) estimated the average effective length of life of adult flies of Drosophila melanogaster in a Bennett population cage to be limited from two to three days.

RESULTS OF FERTILITY OF AMYLASE GENOTYPES

The average number of progeny produced for the amylase types within the population groups and the analysis of variance are presented by Table XV.

TABLE XV

AVERAGE NUMBER OF PROGENY AND ANALYSIS OF VARIANCE FOR THE AMYLASE GENOTYPES IN THE POPULATIONS

ORIGIN OF THE CHROMOSOMES	AMYLASE GENOTYPES							
	AMY^1/AMY^1		$AMY^1/AMY^{1,3}$		$AMY^{1,3}/AMY^{1,3}$		TOTAL	
	N	$\bar{X}$	N	$\bar{X}$	N	$\bar{X}$	N	$\bar{X}$
T/T	72	222.30	192	248.86	81	265.30	345	247.18
W/W	133	251.70	258	278.43	95	290.09	486	273.40
T/W	172	238.31	292	252.64	189	250.15	653	248.15
TOTAL	377	239.98	742	260.63	365	263.90	1484	256.19

ANALYSIS OF VARIANCE

SOURCE	S.S.	d.f.	M.S.	F
Amylase types	151,969	2	75,984	4.19*~**
Populations	230,655	2	115,328	6.36***
Interaction	38,647	4	9,662	0.53
Between genotypes within subclass	2,701,498	149	18,131	2.82***
Within Chromosomes	8,482,451	1326	6,436	

*~**: $p < 0.025$ ***: $p < 0.005$

From Table XV, it is observed that the $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ has a significantly higher fertility than $\underline{Amy}^{1}/\underline{Amy}^{1}$. The average fertility of the heterozygote is intermediate, but it is considerably nearer to the $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ homozygote than to the $\underline{Amy}^{1}/\underline{Amy}^{1}$. The analyses of variance making the comparisons of 1/1 versus 1/1,3 and 1/1,3 versus ^{1,3}/1,3 were then conducted and the results are presented by Table XVI.

TABLE XVI

ANALYSIS OF VARIANCE

COMPARING THE FERITLITY OF AMYLASE GENOTYPES

COMPARISON OF AMY^{1}/AMY^{1} WITH $AMY^{1}/AMY^{1,3}$

SOURCE	S.S.	d.f.	M.S.	F
Amylase genotypes	112,575	1	112,575	6.11*⌣**
Populations	129,820	2	64,910	3.52*
Interaction	9,295	2	4,648	0.25
Between genotypes in subclass	2,153,953	117	18,410	2.84***
Within chromosomes	6,440,531	996	6,466	

*⌣**: $p < 0.025$ ***: $p < 0.005$ *: $p < 0.05$

COMPARISON OF $AMY^{1}/AMY^{1,3}$ WITH $AMY^{1,3}/AMY^{1,3}$

SOURCE	S.S.	d.f.	M.S.	F
Amylase genotypes	8,845	1	8,845	0.56
Population	195,731	2	97,866	6.15***
Interaction	16,703	2	8,352	0.52
Between genotypes in subclass	1,926,117	121	15,918	2.47***
Within chromosomes	6,307,523	980	6,436	

***: $p < 0.005$

These results confirm that the significant difference is between Amy^{1}/Amy^{1} and $Amy^{1}/Amy^{1,3}$ in regard to amylase genotypes. This situation is the same as the results in regard to the k_{σ} values among the amylase genotypes for non-lethals observed in the Cy/+ x Cy/+ viability study.

From examining the population averages pooled over the amylase genotypes in the last column on Table XV, it is evident that the average fertility of the "interpopulational combinations" is very nearly the same as the lowest Texas average, both of which are considerably lower than the Wisconsin average. This was supported by the analysis of variance which demonstrated that the W/W population was significantly higher in fertility than the W/T population, but there was no significant difference between W/T and T/T populations.

COMPARISON OF AMY^1 WITH $AMY^{2,3}$

Although the number of chromosomes (seven) involving the $Amy^{2,3}$ genotype was rather small, they were compared with the $\underline{Amy}^1/\underline{Amy}^1$ genotype. The average number of progeny produced is presented by Table XVII.

TABLE XVII

AVERAGE NUMBER OF PROGENY FOR AMYLASE GENOTYPES INVOLVING AMY^1 AND $AMY^{2,3}$

ORIGIN OF THE CHROMOSOMES	AMYLASE GENOTYPES							
	AMY^1/AMY^1		$AMY^1/AMY^{2,3}$		$AMY^{2,3}/AMY^{2,3}$		TOTAL	
	N	$\bar{X}$	N	$\bar{X}$	N	$\bar{X}$	N	$\bar{X}$
T/T	72	222.30	30	198.77	-	-	102	215.38
W/W	133	251.70	142	224.63	48	183.23	323	229.62
T/W	172	238.31	87	245.01	18	238.83	277	240.86
TOTAL	377	239.98	259	228.48	66	198.39	702	231.99

From these averages it may be observed that the $\underline{Amy}^1/\underline{Amy}^1$ has the highest fertility followed by $\underline{Amy}^1/\underline{Amy}^{2,3}$ and $\underline{Amy}^{2,3}/\underline{Amy}^{2,3}$ respectively. An analysis of variance indicated that these differences were not significant; however, the number of $\underline{Amy}^{2,3}$ chromosomes tested and the number of $\underline{Amy}^{2,3}$ x $\underline{Amy}^{2,3}$ matings were so small that little significance should be attached to this observation.

SUMMARY OF THE RESULTS OF THE FERTILITY STUDY

1. The highest fertility was shown by the $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ genotype, followed by the $\underline{Amy}^{1}/\underline{Amy}^{1,3}$ heterozygote and the $\underline{Amy}^{1}/\underline{Amy}^{1}$ homozygote respectively. The amylase heterozygote was closer to the $\underline{Amy}^{1,3}$ homozygote (no significant difference) than it was to the $\underline{Amy}^{1}$ homozygote (significant difference).

2. There was a highly highly significant difference between population groups with the Wisconsin population being much higher than the Texas and the interpopulational group (the last two are nearly identical), but there was no significant interaction between amylase genotypes and population groups. There was no evidence of heterosis.

CHAPTER V

DEVELOPMENTAL TIME

The developmental rate, as well as the viability and fertility, is one of the important components of fitness. The principal goal of this experiment was to determine any differences in the developmental rate among the amylase genotypes. The three amylase genotypes tested were $\underline{Amy}^1/\underline{Amy}^1$, $\underline{Amy}^1/\underline{Amy}^{1,3}$, and $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ with the origins of the chromosomes for each of these amylase genotypes being T/T, W/W, and T/W.

This study was conducted using the standard sugar containing media and the special starch media, both of which have been previously described in Chapter I. The purpose of employing two different types of culture media was to test for different reactions in developmental time for the amylase genotypes in the two kinds of food.

MATERIALS AND METHODS

The matings to produce the necessary genotypes for the developmental study were $\underline{Cy}/\underline{Amy}^1$(or 1,3) ♀♀ x $\underline{Cy}/\underline{Amy}^1$(or 1,3) ♂♂. These crosses involved 30 $\underline{Amy}^1$ and 32 $\underline{Amy}^{1,3}$ chromosomes from the two populations. Crosses which resulted in complete homozygosity for the second chromosome were excluded and no reciprocal matings were made. A total of 745 chromosomal combinations (for the sugar media) were tested.

After three or four days from the time the appropriate matings ($\underline{Cy}/+$ x $\underline{Cy}/+$) were made, the fertilized females were transferred into a fresh vial. At the same time two or

three fertilized cn bw females from a stock culture were also put into the vial with the Cy/+ females. These females were allowed to oviposit for twenty-four hours and were then discarded. All of the emerging +/+ and cn bw progeny were counted. Since the male, in general, develops a little slower than the female, the progeny were counted separately into the two sexes. The offspring were counted twice daily at 10:00 A.M. and 4:00 P.M. with the first count being made on the afternoon of the ninth day after the mating was made and the final check was made on the afternoon of the thirteenth day. The majority of the flies emerged on the tenth and eleventh day with usually no flies at all on the first and final check. In most of the cultures the four phenotypic types of flies (+/+ males, +/+ female, cn bw male, and cn bw female) were scored, but if any of these four were missing, the culture was eliminated from any further calculations.

One purpose of including the cn bw flies in the developmental study is to bring about more uniform media conditions for larval growth and development. The Cy/+ x Cy/+ mating is not very fertile, but the addition of cn bw females results in more larva (but still not overcrowded) in the culture vials. The presence of a greater number of larva tends to minimize bacterial contamination which in turn leads to better and more uniform food conditions.

The principal reason for including the cn bw females is to have a standard genotype in each culture since it has

previously been observed that the developmental rate is very sensitive to slight environmental changes (temperature, crowding condition, etc.), and it is therefore desirable to have a standard genotype in the culture with which the developmental time of the genotype in question may be compared. From previous observations in the laboratory, it was known that cn bw flies grow, in general, a little slower than the +/+ flies. This difference, measured in hours, is defined as D (developmental time for cn bw - developmental time for +/+) and is computed separately for the male and female progeny in each culture vial. After $D_{\sigma^{\prime}}$ and D_{φ} were calculated, the unweighted averages of the D values of the two sexes were calculated. The value of D, thus computed, was used as a measure of the developmental rate for the different genotypes. Obviously, the larger the value of D, the faster is the development of the +/+ genotype in question. The developmental rate experiments were conducted during about a two months period, and the absolute value of the developmental time fluctuated from time to time, but the D values remained somewhat constant.

COMPARISON OF AMY^{1} AND $AMY^{1,3}$ CHROMOSOME COMBINATIONS FOR THE SUGAR MEDIA

The average D values and analysis of variance are given by Table XVIII.

TABLE XVIII

AVERAGE $\underline{D}$ VALUES AND ANALYSIS OF VARIANCE FOR AMY^1 AND $AMY^{1,3}$ GENOTYPIC COMBINATIONS FOR THE SUGAR MEDIA

	AMYLASE GENOTYPES							
	AMY^1/AMY^1		$AMY^1/AMY^{1,3}$		$AMY^{1,3}/AMY^{1,3}$		TOTAL	
POPULATIONS	$\overline{D}$	N	$\overline{D}$	N	$\overline{D}$	N	$\overline{D}$	N
W/W	9.68	54	9.68	122	8.20	58	9.31	234
T/T	4.69	38	7.78	99	8.20	53	7.28	190
W/T	7.46	77	8.81	158	8.23	86	8.33	321
TOTAL	7.55	169	8.82	379	8.21	197	8.37	745

ANALYSIS OF VARIANCE

SOURCE	S.S.	d.f.	M.S.	F
Amylase genotypes	205.86	2	102.93	2.16
Populations	441.58	2	220.79	4.63**
Interaction	310.49	4	77.62	1.63
Between genotypes within subclass	35,072.16	736	47.65	

**: $p < 0.01$

From this table it is observed that there is no significant difference in $\underline{D}$ among the amylase genotypes, but there is a highly significant difference between the population groups.

LETHAL AND NON-LETHAL COMBINATIONS

From a total of 62 chromosomes, there were 11 which carried recessive lethals (6 for Amy^1 and 5 for $Amy^{1,3}$). There was a total of 238 matings which involved these lethals and 507 matings not involving lethals. The average D values for the lethals and non-lethals were nearly the same, i.e., 8.33 and 8.39 respectively. The analysis of variance was made for the lethals and non-lethals separately; the results were essentially the same as given by Table XVIII with the lethal and non-lethal classes each showing the same tendency as the pooled results.

COMPARISIONS OF AMY^1 AND $AMY^{1,3}$ CHROMOSOME COMBINATIONS FOR THE STARCH MEDIA

A total of 10 Amy^1 and 12 $Amy^{1,3}$ chromosomes from the Texas and Wisconsin populations were tested in the starch media. Except for the difference in the composition of the media, this experiment was carried out as previously described in this Chapter of the 22 chromosome lines there were 6 recessive lethals (3 in the Amy^1 lines and 3 in the $Amy^{1,3}$ lines). There were a total of 116 matings involving non-lethal chromosomes and 101 involving lethals for which the averages D values were 6.53 and 6.05 respectively. This difference between non-lethals and lethals was not significant.

The analysis of variance for the lethal and non-lethal classes was performed separately with no significant

difference between amylase genotypes and no significant interaction between amylase types and populations, but there was a significant difference between populations. Since the difference between lethals and non-lethals was not significant, the data was pooled. The average $\underline{D}$ values and analysis of variance of the pooled data are shown by Table XIX.

From Table XIX, a significant difference was observed among the amylase genotypes with the $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ having the fastest development.

TABLE XIX

AVERAGE $\underline{D}$ VALUES AND ANALYSIS OF VARIANCE FOR AMY^1 AND $AMY^{1,3}$ GENOTYPIC COMBINATIONS FOR THE STARCH MEDIA

	AMYLASE GENOTYPES							
	AMY^1/AMY^1		$AMY^1/AMY^{1,3}$		$AMY^{1,3}/AMY^{1,3}$		TOTAL	
POPULATIONS	$\overline{D}$	N	$\overline{D}$	N	$\overline{D}$	N	$\overline{D}$	N
W/W	7.41	14	9.14	34	11.90	15	9.41	63
T/T	2.54	6	4.94	23	3.59	14	4.17	43
W/T	3.15	24	5.09	57	7.70	30	5.38	111
TOTAL	4.42	44	6.27	114	7.79	59	6.31	217

ANALYSIS OF VARIANCE

SOURCE	S.S.	d.f.	M.S.	F
Amylase genotypes	359.00	2	179.50	3.30*
Populations	971.99	2	486.00	8.94***
Interaction	33.29	4	8.32	0.15
Between genotypes within subclass	11,310.87	208	54.38	

*: $p < 0.05$ ***: $p < 0.005$

$\underline{Amy}^1/\underline{Amy}^1$ having the slowest, and the $Amy^1/Amy^{1,3}$ heterozygote being intermediate. The differences among populations were highly highly significant with the Wisconsin population being much faster in development than either the Texas (slowest) or the interpopulational group.

COMPARISON OF SUGAR AND STARCH MEDIA

The overall average D value for the sugar media was 8.37 in comparison with an overall average of 6.31 for the starch media. This difference was highly highly significant with the sugar media giving a faster developmental time. The same results are also true if the data is separated into lethal and non-lethal classes.

The number of matings and averages for the subclasses of the amylase genotypes and the populations for the starch and sugar media is given by Table XX.

The number of matings and chromosome combinations in the starch media was small, but it is interesting to notice that the overall averages (now totals) indicate that the

$\underline{Amy}^1/\underline{Amy}^1$ and $\underline{Amy}^1/\underline{Amy}^{1,3}$ are more strongly influenced by the change of media than $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$. In addition, the Wisconsin population, which showed the fastest development among the three groups, appeared to be much less affected by the change in food media (compare column totals) than the other two populational groups.

TABLE XX

NUMBER OF MATINGS AND AVERAGE D VALUES

FOR SUGAR AND STARCH MEDIA

ORIGIN OF		AMYLASE GENOTYPE							
		AMY^1/AMY^1		$AMY^1/AMY^{1,3}$		$AMY^{1,3}/AMY^{1,3}$		TOTAL	
CHROMOSOMES		N	$\overline{D}$	N	$\overline{D}$	N	$\overline{D}$	N	$\overline{D}$
W/W	SUGAR	54	9.68	122	9.68	58	8.20	234	9.31
	STARCH	14	7.41	34	9.14	15	11.90	63	9.41
T/T	SUGAR	38	4.69	99	7.78	53	8.20	190	7.27
	STARCH	6	2.54	23	4.94	14	3.59	43	4.16
W/T	SUGAR	77	7.46	158	8.81	86	8.23	321	8.33
	STARCH	24	3.15	57	5.09	30	7.70	111	5.37
TOTAL	SUGAR	169	7.55	379	8.82	197	8.21	745	8.37
	STARCH	44	4.42	114	6.27	59	7.79	217	6.31
GRAND TOTAL		213	6.90	493	8.23	256	8.12	962	7.91

Further analyses of variance were perfomed for each amylase genotype over the three population groups in the two types of media. For the $\underline{Amy}^1/\underline{Amy}^1$ and $\underline{Amy}^1/\underline{Amy}^{1,3}$ amylase genotypes, there was a significant difference between media and also between the population groups. As may be observed

from Table XX, the starch media always gave slower development than the sugar and the Wisconsin population always gave the fastest development followed by the interpopulational group and the Texas population. This same tendency for the population comparisons was also true for the $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ genotype, but the differences observed between the media were rather irregular. For this analysis of the $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ genotype the interaction term was significant at the 5% level indicating a different response between the Wisconsin and Texas populations with regard to media. In conclusion it would appear that the faster developing genotypes are less affected than the more slowly developing ones by the change in the media.

SUMMARY OF RESULTS OF THE DEVELOPMENTAL STUDY

1. In the starch media the amylase genotypes exhibited a significant difference in developmental time with $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ having the fastest developmental time followed by $\underline{Amy}^{1}/\underline{Amy}^{1,3}$ and $\underline{Amy}^{1}/\underline{Amy}^{1}$ respectively. A similar trend (although not significant) was observed in the sugar media with the exception of $\underline{Amy}^{1}/\underline{Amy}^{1,3}$ being slightly faster than $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$.

2. In both sugar and starch media, the Wisconsin group developed fastest, the Texas group slowest, and the interpopulational group was intermediate.

3. The flies generally developed faster in the sugar media than in the starch media.

4. Although the number of chromosome combinations for the starch media is rather small, the results of the media comparison suggests that the amylase genotypes which have the faster developmental rates are less influenced by changing the media composition than those which develop more slowly.

CHAPTER VI

DISCUSSION OF RESULTS AND POSSIBLE MECHANISMS FOR MAINTAINING THE AMYLASE POLYMORPHISM

FURVEY OF NATURAL POPULATIONS

The survey of the flies collected from the natural populations of Texas and Wisconsin revealed that the amylase polymorphism consists primarily of the $\underline{Amy}^{1}$ and $\underline{Amy}^{1,3}$ alleles. The frequency of these two alleles was somehow similar in both populations with an overall average gene frequency of about 0.80 for $\underline{Amy}^{1}$ and 0.12 for $\underline{Amy}^{1,3}$. Other less frequent alleles observed in the survey included $\underline{Amy}^{1,2}$, $\underline{Amy}^{2,3}$, and $\underline{Amy}^{1,6}$. These alleles also were found in somewhat similar frequencies in both populations with the exception of $\underline{Amy}^{2,3}$ being considerably more common in the Wisconsin population.

The results of the Odate "semi-natural" population were in general agreement in regard to the alleles observed with the Texas and Wisconsin populations. From the Odate population, five lines out of 116 lines checked were observed segregating for two alleles. Because these flies had been maintained in the laboratory in small populations over a period of four years, most of the variation (about 99%) would have been expected to be lost by chance alone. Since nearly 5% were still segregating for two alleles, this strongly suggests that some sort of selection is occurring in order to maintain the polymorphism.

Originally it was intended to obtain information on the viability, fertility, and developmental rate for all the amylase alleles observed in the Texas and Wisconsin populations, and many chromosome lines maintaining these alleles were established. Some preliminary studies for the fertility of the $\underline{Amy}^{2,3}$ allele and some viability studies for the $\underline{Amy}^{1,2}$ allele were conducted; however, it was concluded that there was insufficient time and not really enough chromosome lines to obtain any very definite conclusions regarding these rarer alleles. For this reason the remainder of this study focused attention upon the $\underline{Amy}^{1}$ and $\underline{Amy}^{1,3}$ alleles.

VIABILITY STUDY: GENERAL COMMENTS

As was previously discussed in the sex ratio study in the third chapter, there is some question regarding the degree of dominance of the Cy chromosome over the various + chromosome homologues in the Cy/+ flies. It is uncertain how large an error this factor might cause in the Cy/+ Cy/+ study. Since the results of both the backcross and Cy studies are similar in regard to the viability of the amylase genotypes, and since the backcross study employs a standard (cn bw) which is independent of the chromosome being tested, the possible fluctuations in the degree of dominance of the Cy chromosome do not seem likely to cause a serious error in the viability estimates.

RESULTS OF VIABILITY STUDY

In both viability studies, the k values were subdivided

into $k\sigma^{\male}$ for the male progeny and $k^{\female}$ for the female progeny in order to learn if the k values differed according to sex. The overall $k^{\female}$ value was significantly higher in the Cy/+ x Cy/+ study, but it was significantly lower in the backcross study. Of perhaps more interest for this investigation was the fact that the $k\sigma^{\male}$ values demonstrated a much wider range of variation in both experiments with regard to amylase genotypes than the $k^{\female}$ values did. The reason for this greater variation in the male progeny is not clear at present; however, it is important to observe that the $k^{\female}$ values exhibited the same tendency as those of the $k\sigma^{\male}$. Because of the greater variation of the male progeny, the significant differences in regard to amylase genotypes were found in the male progeny but not in the female progeny.

The k values for the amylase genotypes were quite consistent in both of the viability experiments. The $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ had the highest k value followed by $\underline{Amy}^{1}/\underline{Amy}^{1,3}$ and $\underline{Amy}^{1}/\underline{Amy}^{1}$ respectively. The backcross experiment, which produced no $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ genotypes also demonstrated a significantly higher k value for the $\underline{Amy}^{1}/\underline{Amy}^{1,3}$ in comparison with the $\underline{Amy}^{1}/\underline{Amy}^{1}$ genotype. The difference in k values (male and female pooled together) is roughly 1.5 percent between the $\underline{Amy}^{1}/\underline{Amy}^{1}$ and $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ genotypes with the heterozygote being intermediate.

Since the differences between population groups and differences between lethal versus non-lethals are of second-

ary interest in this study, the results will be only briefly discussed. There were no significant differences between populations in the Cy/+ x Cy/+ study. On the other hand, the backcross study indicated a higher k value for the Wisconsin population in comparison with the Texas population. The number of matings involving recessive lethal second chromosomes was relatively small in comparison to those involving non-lethals; nevertheless, the lethals generally demonstrated a lower k value (significantly lower in the Cy/+ x Cy/+ study) than the non-lethal matings. Finally it should be stressed that no significant interactions were observed either between the amylase genotypes and populations or the amylase genotypes and lethal versus non-lethal classes, thus indicating the amylase genotypes acted in a consistent manner in both the population groups and the lethal and non-lethal classes.

FERTILITY EXPERIMENT

The purpose of the fertility experiment was to test for the number of progeny produced by the amylase genotypes in the population groups. No chromosomes containing lethals were included.

The lowest fertility was shown by the Amy^{1}/Amy^{1} genotype with a significantly higher fertility of the $Amy^{1}/Amy^{1,3}$ heterozygote, and the highest fertility of the $Amy^{1,3}/Amy^{1,3}$ homozygote. If a value of 1.0000 is assigned to the $Amy^{1,3}/Amy^{1,3}$ genotype, the relative values of

$\underline{Amy}^1/\underline{Amy}^{1,3}$ and $\underline{Amy}^1/\underline{Amy}^1$ are 0.9876 and 0.9094 respectively. From this it may be concluded that the $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ has about 9% greater fertility than the $\underline{Amy}^1/\underline{Amy}^1$ with the heterozygote being intermediate although somewhat closer to the more fertile homozygote.

The population groups also demonstrated significant fertility differences with the Wisconsin population being significantly higher than the other two. It is interesting to observe that the interpopulational group is nearly the same as the Texas population. This strongly indicates no evidence of heterosis in regard to fertility even when the chromosome combinations were from two widely separated areas and would presumably be heterozygous for as large or even larger number of loci than those matings within populations. The interaction between the populations and amylase genotypes was not significant which indicated (as it did in the viability experiment) a consistent behavior of the amylase genotypes in the three population groups.

DEVELOPMENTAL STUDY

From the developmental study it may be concluded that $\underline{Amy}^1/\underline{Amy}^1$ has the slowest developmental time in both the sugar and starch media, although this difference is not significant for the sugar media. The heterozygote has a very slightly faster development than the $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ homozygote in the sugar media, whereas the homozygote has a faster development in the starch media. Although the

evidence is not quite as clear-cut as in the viability and fertility studies, it is evident that the $\underline{Amy}^1/\underline{Amy}^1$ has the slower rate of development in comparison with the other two amylase genotypes.

The populations behaved just as in the fertility experiment with the Wisconsin population having the fastest developmental time with the interpopulational group and Texas population following respectively with slower growth rates. The crosses involving lethals was rather small and was slightly slower in developing than the non-lethals.

MECHANISMS FOR MAINTAINING POLYMORPHISMS

From the results of the viability, fertility, and developmental studies on the amylase genotypes, it is difficult to understand how the Amy^1 allele is maintained in such a high frequency in the natural populations. Of course, one easy explanation is that the amylase polymorphism is transient and the $\underline{Amy}^{1,3}$ allele is in the process of replacing the Amy^1 allele. On the other hand, this possibility is difficult to reconcile with the frequency of $\underline{Amy}^1$ and $\underline{Amy}^{1,3}$ being similar in two widely separated populations. The frequency of the Odate population was also somehow similar to the Texas and Wisconsin populations with $\underline{Amy}^1$ and $\underline{Amy}^{1,3}$ still segregating in two of the Odate cultures and only one being fixed for $\underline{Amy}^{1,3}$.

If a stable balanced polymorphism is assumed, the central problem which still remains to be solved is to try

to discover some compensating mechanism giving some selective advantage for the $\underline{Amy}^{1}$ allele. The various mechanism for maintaining a polymorphism are given in a report by Frydenberg (1963) and will be briefly discussed in relationship to the findings of this present study.

1. Heterosis. One of the most frequent explanations for maintaining a stable equilibrium is through overdominance in fitness of the heterozygote (in this case $\underline{Amy}^{1}/\underline{Amy}^{1,3}$) over the homozygotes. The results of this study eliminate this possibility because the heterozygote was nearly always intermediate in fitness and was never significantly higher than both homozygotes.

On the other hand, it might be noted that the $\underline{Amy}^{1,3}$ allele is considered a duplication and if each isozyme band functioned slightly differently physiologically, the duplication might give the $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ homozygote the greater flexibility that is often given as an explanation for the heterotic effect of the heterozygote. In an example such as this the fitness of the $\underline{Amy}^{1}/\underline{Amy}^{1,3}$ and $\underline{Amy}^{1,3}/\underline{Amy}^{1,3}$ would be expected to be nearly equal with the $\underline{Amy}^{1}/\underline{Amy}^{1}$ having a considerably lower fitness. Unfortunately very little is known about how these amylase isozymes function except that they break down glycogen in the fly.

2. Alternative alleles favored in different niches. This mechanism was reported by Leven (1953) and indicates that the equilibrium may be stable without the heterozygote being superior in any one niche. This explanation seems unlikely

for the present study since the frequencies of the alleles were basically similar in all the populations tested. In addition, alternative alleles were not favored in the two types of media used in the developmental study.

3. Alternative alleles favored in the two sexes. Haldane (1962) presented a short report which demonstrated that a stable equilibrium could be maintained if alternative alleles were favored in the two sexes. The viability experiment demonstrated a wider range of k values of the amylase genotypes in the male progeny, but the female progeny showed the same tendency as the male progeny. This makes this model unlikely.

4. Gene frequency dependent selective values. The three previous explanations for maintaining polymorphisms assume the selective value of the different genotypes to be independent of the gene frequency. The difference in this mechanism is that the selective values change as the gene frequencies change. This model has recently been proposed for maintaining the Esterase 6 (Yarbrough and Kojima, 1967) and the alcohol dehydrogenase (Kojima and Tobari, 1969) polymorphism in Drosophila melanogaster. The principal is that once the population reaches the equilibrium frequency, the two isozyme alleles become nearly selectively neutral. If the gene frequency of one of the alleles becomes greater than the other when at the equilibrium frequency, the respective genotypes are at a selective disadvantage; if the

frequency is lower the genotypes have a selective advantage. In this manner the genes are maintained at a stable frequency without it being necessary to have heterozygote superiority.

The report of Lewontin and Hubby (1966), which dealt with the amount of heterozygosity in a survey of enzymes and proteins in some natural populations of Drosophila pseudoobscura, estimated the average population to be polymorphic at 30% of all loci with an average of 12% of the individuals genome being heterozygous. If heterosis was considered the mechanism for maintaining this large number of polymorphic loci, some way must be found to reduce the rather large segregation load due to the inferior homozygotes. The frequency selection dependent model gets around this problem by having the two alleles nearly selectively neutral at the equilibrium frequency.

This mechanism was not tested in the present study, but some information may be obtained from the viability backcross study. In this experiment the frequency of $\underline{Amy}^1$ was 0.75 which is approximately equal to (slightly lower than) its frequency observed in the survey of the natural populations. If the natural populations are assumed to be in equilibrium, it might be expected for the two alleles to be selectively neutral, but the k value for the $\underline{Amy}^1/\underline{Amy}^{1,3}$ genotype was significantly higher than the $\underline{Amy}^1/\underline{Amy}^1$ genotype. Experiments for testing the gene frequency dependent selective values

are planned in the near future for the amylase polymorphism.

5. Prezygotic selection. In this study, as well as in many other studies, the viabilities of the genotypes have been estimated by comparing the relative frequencies of genotypes, or the k values, recovered among the progenies of the appropriate matings. These estimations were made based on the assumption that the heterozygous parents produce two kinds of functional gametes in the frequency of 50% throughout their reproductive life span, and therefore any deviation from the expected frequency is due to differential viabilities of genotypes. Could this be an acceptable assumption?

In 1969, Hiraizumi and Grove showed, for the recessive lethals isolated from a Tokyo wild laboratory stock, that the average k value for the lethals as compared with the lethal-free controls was dependent upon the age of the parental males; it decreased slightly but consistently when the lethal heterozygous males got old. When the k value was computed based on the progenies produced by the younger males, the average k for the lethals was significantly higher than that for the controls while the progenies produced by the older males gave lower k values for the lethals. This result is very difficult to explain by a simple differential viability model, and has to be due to selection which operates prior to the zygotic stage.

The paternal age dependent segregation frequency has

been demonstrated to exist in the Segration Distorter system of Drosophila melanogaster (Sandler and Hiraizumi, 1961; Hiraizumi and Watanabe, 1969), and in the Bar-Stone system in D. melanogaster (Zimmaring and Barbour, 1961). This phenomenon has been termed as "aging effect on segregation frequency" or simply "aging effect". The sex ratio in Drosophila (Yanders, 1965), and in man (Novitski and Sandler, 1956) was also found to be subject to the aging effect.

Fortunately, in the present study, the Cy/+ x Cy/+ viability experiment was designed, although not in a critical way, to make it possible to study the relationship between the value of k and the parental age. As was described in Chapter 3, the age of the parental flies in this experiment was less than 1.5 days old at the time of mating, and in every 4 days the parental flies were transferred to fresh, new culture vials. The average age of the parents for the 1st, 2nd and the 3rd age period was, taking the middle point of each interval, 2.75, 6.75 and 10.75 days respectively. The $k_{\sigma^{\prime}}$ and k_{φ}^{+} values were computed for the 16 mating types based on the pooled number (for computational simplicity) of progeny flies for each mating type. These results are summarized by Table XXI.

TABLE XXI

THE LIST OF $k\sigma^{\male}$ AND $k\varphi$ VALUES FOR EACH PARENTAL AGE PERIOD

$k\sigma^{\male}$

Amylase mating type ♀ ♂	Average ages of parents in days 2.75	6.75	10.75
Amy^{1}/Cy x Amy^{1}/Cy	0.385	0.337	0.372
$Amy^{1,3}/Cy$ x Amy^{1}/Cy	0.392	0.359	0.371
Amy^{1}/Cy x $Amy^{1,3}/Cy$	0.382	0.360	0.351
$Amy^{1,3}/Cy$ x $Amy^{1,3}/Cy$	0.376	0.373	0.372
TOTAL	0.384	0.357	0.367

$k\varphi$

Amy^{1}/Cy x Amy^{1}/Cy	0.382	0.370	0.367
$Amy^{1,3}/Cy$ x Amy^{1}/Cy	0.393	0.373	0.366
Amy^{1}/Cy x $Amy^{1,3}/Cy$	0.388	0.367	0.365
$Amy^{1,3}/Cy$ x $Amy^{1,3}/Cy$	0.372	0.360	0.380
TOTAL	0.384	0.368	0.370

As can be seen by Table XXI, the $\underline{k}$ values were different among the parental age periods, and this change was stitistically significant for both $k\sigma^{\male}$ and $k\varphi$ ($p < 0.005$ and $p < 0.05$ respectively). The $\underline{k}$ value seems to stay the same among the age periods for the 1,3 x 1,3 mating, but a large

decrease in k can be seen for the 1 x 1 mating. The reciprocal matings, 1,3 x 1 and 1 x 1,3, appear to have somewhat similar aging patterns and are intermediate between the above two mating types. This suggests that both parents are responsible for the aging effect. It is noted that both k_{o}^{σ} and k_{+}^{φ} show the aging effects, but the effect is less pronounced in the k_{+}^{φ}. It is also noted that the k_{o}^{σ} and k_{+}^{φ} values are the same for the youngest age period, but k_{+}^{φ} becomes larger than k_{o}^{σ} at the later age period, thus suggesting that the difference between k_{+}^{φ} and k_{o}^{σ} (see Chapter 3) is a reflection of the aging effect.

The relationship between the age of parents and the k value for the 1 x 1 mating does not seem to be linear. It decreases during the 1st week period, but then later it tends to increase. A similar situation, a periodic change in k, was reported by Hiraizumi and Watanabe (1969) for the Tokyo/cn bw heterozygous males.

The reproductive life span of Drosophila melanogaster in natural populations is not yet well understood, but the effective length would certainly be rather short. Regardless of the life span of flies in nature, however, the aged flies would be of less consequence to the total fitness with the major contribution to the fitness coming from the younger age period. In this respect it is interesting to examine the k values for the younger parental age periods such as the 1st and the 2nd age groups (2.75 and 6.75 days old).

Although the relationship between the k value and the parental age may not be linear, it may be approximated with linear regression. The linear regression coefficient, b, for each mating type, k_{σ} and k_{φ} separately, is listed by Table XXII, and the variance analysis for the comparison of b among amylase mating types for k_{σ} by Table XXIII.

TABLE XXII

REGRESSION COEFFICIENTS (b) OF k_{σ} AND k_{φ}

FOR THE FIRST TWO AGE GROUPS

k_{σ}

Genotype of ♀ parent	Genotype of ♂ parent Amy^{1}/Cy	Genotype of ♂ parent $Amy^{1,3}/Cy$
Amy^{1}/Cy	b= - 0.01200	b= - 0.00544
$Amy^{1,3}/Cy$	b= - 0.00825	b= - 0.00069

k_{φ}

Genotype of ♀ parent	Genotype of ♂ parent Amy^{1}/Cy	Genotype of ♂ parent $Amy^{1,3}/Cy$
Amy^{1}/Cy	b= - 0.00306	b= - 0.00538
$Amy^{1,3}/Cy$	b= - 0.00500	b= - 0.00306

TABLE XXIII

ANALYSIS OF VARIANCE FOR THE HOMOGENEITY IN b FOR THE $k\sigma^{\nearrow}$

Source of variation	S.S.	d.f.	M.S.	F
Between male genotypes	0.001590	1	0.001590	12.14**
Between female genotypes	0.000578	1	0.000578	4.41*
Interaction	0.000024	1	0.000024	0.18
Error	0.003136	24	0.000131	

*: $p < 0.05$ **: $p < 0.01$

From Table XXIII it may be seen that the difference in b was significant between male genotypes and between female genotypes and the interaction term was not significant. The values of b for 1 x 1,3 and 1,3 x 1 were approximately 50% of that for 1 x 1 whereas the b for 1,3 x 1,3 was practically zero. From these considerations it may be concluded that the aging effect, at least for the first nine day period, is greatest for the 1 x 1 mating type, less for the 1 x 1,3 and 1,3 x 1 mating types, and practically nothing for the 1,3 x 1,3 mating type.

The same variance analysis was made for the female progeny ($k\overset{\circ}{+}$), but none of the differences was significant. This indicated that there was not much difference among the four amylase mating types.

In the present experiment, the progeny flies produced

by the parents more than 0.75 but less than 4.75 days old were pooled together into the 2.75 days old group. The decrease in k value could occur, of course, within this age interval. Hiraizumi and Watanabe (1969) showed that a considerable amount of aging effect could occur even within a few days period; e.g., the average k value for the Tokyo/cn bw males was 0.570 when the males were 0.5 days old, 0.541 for 1.5 days old, and 0.516 for 2.5 days old. It is interesting to estimate the k values for the very young parents, say 1 day old. These are computed, by using the regression coefficient listed in Table XXII. The estimated k values are shown by Table XXIV.

TABLE XXIV

ESTIMATE OF k VALUES FOR ONE DAY OLD PARENTS

Mating	k(♂)	k(♀)	Average
1 x 1	0.406 ± 0.013	0.388 ± 0.016	0.397 ± 0.010
1,3 x 1	0.407 ± 0.009	0.405 ± 0.012	0.406 ± 0.007
1 x 1,3	0.391 ± 0.008	0.400 ± 0.009	0.395 ± 0.006
1,3 x 1,3	0.378 ± 0.014	0.378 ± 0.011	0.378 ± 0.009

The present study does not provide any information on the mechanism of the aging effect or on prezygotic selection. This must wait for future studies. Nevertheless, it appears that the Amy^1 allele from natural populations, when made heterozygous with Cy, shows a fairly strong aging effect;

its recovery rate among the offspring decreases (for the first 9 day period at least) when the parental flies get old. On the other hand, the recovery rate of the $\underline{Amy}^1$ among offspring tends to become higher than that of the $\underline{Amy}^{1,3}$ allele for the very young parents. It may therefore be concluded that prezygotic selection is in favour of the $\underline{Amy}^1$ allele when the parents are very young.

Based on the results from the fertility and developmental time studies, it seems reasonable to assume that the zygotic fitness of 1/1,3 heterozygotes is nearly equal to that of the 1,3/1,3 homozygotes. Let us assign the zygotic fitness for these genotypes equal to 1, and let the fitness of 1/1 homozygotes be W_{11}. Assume prezygotic selection is operating in the two sexes and let $\underline{C}$ be the frequency of $\underline{Amy}^1$ allele among functional gametes produced by the 1/1,3 heterozygous parents (if there is no prezygotic selection, $\underline{C} = 0.5$). The equilibrium frequency of the $\underline{Amy}^1$ allele, q, is given by the following formula (Hiraizumi et al, 1961),

$q = \frac{2CW12 - 1}{2W_{12} - W_{11} - 1}$, or if the fitness of the heterozygote (W_{12}) is equal to one, then $q = \frac{2C - 1}{1 - W_{11}}$ . . . . . (1)

and the condition for the stable equilibrium state is given by $2W_{12} - W_{11} - 1 \quad 2CW_{12} - 1 \quad 0$, or $1 - W_{11} \quad 2C - 1 \quad 0$.

The $\underline{C}$ value for the younger age period of flies may be estimated in the following way. Since the age of the parental

flies in the backcross matings was not so strictly regulated as those of the Cy/+ Cy/+ experiment, only the latter will be used to estimate C. The average of ko and k♀+ will be employed as a recovery frequency of the non-Cy phenotype. Since both parents seem to cause the aging effect (reciprocal matings showed a similar aging pattern), the k values for the reciprocal matings of Cy/Amy^{1} ♀ x $Cy/Amy^{1,3}$ ♂ and $Cy/Amy^{1,3}$ + x Cy/Amy^{1}♂ are pooled.

For the general case, let $V_{1.1}$, $V_{1.1,3}$ and $V_{1,3.1,3}$ be the relative viability of 1/1, 1/1,3 and 1,3/1,3 genotype respectively, and let the relative viability of the Cy/+ genotype be 1. Also we may let C be = 0.5 for the Amy^{1}/Cy (prezygotic selection present, and shows aging effect), and C = 0.5 for the $Amy^{1,3}/Cy$ genotype (no prezygotic selection, no aging effect). Then we have

$$k1.1 \text{ for } (1/Cy \times 1/Cy) = \frac{C\ V1.1}{C\ V1.1 + 2(1-C)} \quad - - - - (2)$$

$$k1.1,3 \text{ for } (1/Cy \times 1,3/Cy) = \frac{C\ V1.1,3}{1 + C\ V1.1,3} \quad - - - (3)$$

$$k1,3.1,3 \text{ for } (1,3/Cy \times 1,3/Cy) = \frac{V1,3.1,3}{2 + V1,\ 3\text{-}1,3} \quad - (4)$$

We shall here estimate C and V's based on the youngest age group (=0.75-4.75 days old) on the assumption that this period would play the most important role in populations. From Table XXI, $k_{1.1} = 0.384$, $k_{1.1,3} = 0.389$, and $k_{1,3.1,3} = 0.374$. From equation 4, we obtain $V_{1,3.1,3} = 1.19$. The C values corresponding to the various sets of V values are

presented in Table XXV.

TABLE XXV

THE VALUES OF $\underline{C}$ FOR SEVERAL SETS OF V VALUES

$V_{1,3.1,3}$	$V_{1.1,3}$	$\frac{V_{1.1,3}}{V_{1,3.1,3}}$	$V_{1.1}$	$\frac{V_{1.1}}{V_{1,3.1,3}}$	C
1.19	1.15	0.966	1.004	0.844	0.554
	1.16	0.975	1.024	0.861	0.549
	1.17	0.983	1.045	0.878	0.544
	1.18	0.992	1.062	0.892	0.540
	1.19	1.000	1.084	0.911	0.535
	1.20	1.008	1.106	0.929	0.530
	1.21	1.017	1.124	0.945	0.526

It may be seen from this table that the $\underline{C}$ value lies somewhere between 0.53 to 0.55 for a reasonable range of V values.

If $V_{1.1,3} = V_{1,3.1,3} = V = 1.19$, the values of $\underline{C}$ and $V_{1.1}$ can be obtained by solving the equations (3) and (4) for $\underline{C}$ and $V_{1.1}$. Thus, $\underline{C} = 0.535$, $V_{1.1} = 1.08$. The viability of 1/1 relative to 1/1,3 or 1,3/1,3 is computed to be 1.08/1.19 = 0.91. If we estimate these values based on the 2nd age group, we obtain $\underline{C} = 0.496$, $V_{1,3.1,3} = 1.16$ and $V_{1.1} = 1.10$, 1.10/1.16 = 0.949. Note that instead of a large decrease in $\underline{C}$, the values of V stay somewhat constant which, of course, should be so if the model is appropriate.

The average age of the parents for the backcross experiment was approximately 5 days. If the C value for the cn bw chromosome in the backcross matings is taken as approximately 0.515 (average of the two C values calculated above) and the viability of Amy^{1}/cn bw is taken to be 0.91 relative to $Amy^{1,3}$/cn bw (as was estimated above), the average k value of the $Amy^{1,3}$/cn bw backcross mating would be larger than the Amy^{1}/cn bw matings by approximately 0.01 which, in fact, agrees reasonably well with the actual observation.

The 1/1 genotype females showed, on the average, about a 10% reduction in fertility. No exact test was made to measure the male fertility differences among amylase genotypes, but since, in the +/Cy x +/Cy experiment, the amylase genotype of the parental males was of little consequence to the number of progeny (see Table XIV), it may be safely assumed that the male fertility is nearly the same between the 1/1 and the 1/1,3 (or 1,3/1,3) genotypes. We therefore take the average of the two sexes, i.e., 0.95, as an estimate of fertility of the 1/1 genotype. The value of W_{11} would be then, combining the viability and the fertility, somewhere between 0.85 and 0.90.

Substituting C = 0.535, W_{11} = 0.85 or 0.90 (these satisfy the conditions for stability) into the equation (1), we obtain.

$q = 0.07/0.15 = 0.47$, or $q = 0.07/0.10 = 0.70$

The equilibrium frequency of $\underline{Amy}^1$ allele thus estimated is still lower than that observed in natural populations, but the agreement is reasonably close.

Since the question of how the $\underline{Amy}^1$ allele is maintained in such a high frequency in natural populations has not been fully determined yet, some future experiments concerning this problem are briefly mentioned. A more critical experiment testing for prezygotic selection is indicated from the results concerning the aging effect of the $\underline{Cy}/+ \times \underline{Cy}/+$ viability experiment. The change in $\underline{k}$ as the parental flies get older should be examined over a shorter period of time, (such as every two days) for the different amylase genotypes. Because the SR ratio study indicated possible fluctuations in the degree of dominance of the $\underline{Cy}$ chromosome over the different + chromosomes, a different mating scheme should be employed in which the standard (such as $\underline{Cy}/\underline{L}$) would be independent of the chromosomes being tested.

In addition to this, experiments for testing frequency dependent selection as well as further studies employing the starch containing media are currently being planned. It is hoped that these future experiments will help to clarify why the $\underline{Amy}^1$ allele is the most common in natural populations.

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