STUDIES ON BODY COLOUR POLYMORPHISM IN
DROSOPHILA MELANOGASTER

by

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INTRODUCTION

The classical theory of genetics postulated that genes were sharply divided into two types, those advantageous to the species and those disadvantageous to it. Advantageous genes would be incorporated into the genotype of the species, while disadvantageous ones would be eliminated. Thus genetic variation would be kept at a minimum and the uniformity of the species maintained. Studies on the genetic variation in natural populations have not supported this theory. An increasing amount of variation is being shown to exist in populations, an amount much greater than that predicted by the classical theory.

The occurrence of discontinuous phenotypes within a species is a relatively common phenomenon in both the animal and plant kingdoms. The existence of discontinuous phenotypes within a population due to genes at intermediate frequencies is termed polymorphism. The first definition of polymorphism was put forward by Ford in 1940. He stated that "polymorphism is the occurrence together in the same locality of two or more discontinuous forms of a species in such proportions that the rarest of them cannot be maintained merely by recurrent mutation".

Ford (1940) divided polymorphisms into two types, balanced and transient. In the former type the genes are maintained at intermediate frequencies, whereas, in the latter, the trend is towards fixation of one allele. Balanced polymorphism appears to be the more common phenomenon and is of very widespread occurrence. In some cases, however, it may be difficult to distinguish between the two types unless observations are made over a considerable period of time.

The term 'polymorphism' is possibly a misleading one, as the suffix 'morphism' suggests that the phenomenon is limited to structural characters. This is not the case and 'polymorphism' may be applied to any phenotypic character, whether it is morphological, biochemical, physiological or behavioural, provided it is genetically controlled and the phenotypes have a discontinuous distribution.

The hypothesis that balanced polymorphism is maintained due to the adaptive superiority of the heterozygote was first developed by Muller (1918) and Fisher (1930). They showed theoretically that two alleles, A and a, can be maintained in a population at intermediate frequencies if the fitness of the heterozygote (Aa) is higher than that of either homozygote. This hypothesis was verified experimentally by Wright and Dobzhansky (1946) from studies on populations of *Drosophila pseudoobscura*. In these experiments it was shown that the heterozygotes for certain chromosomal inversions possessed considerable adaptive superiority over the homozygotes.

Most of the work on balanced polymorphism in *Drosophila* has been done using the chromosomal inversions found in a number of *Drosophila* species. In order to determine the inversion types present in a particular individual, it is necessary to use cytological analysis of the salivary gland chromosomes of the larva. Thus the labour involved in the detection and study of chromosomal variants is considerable.

The discovery of balanced polymorphisms affecting body pigmentation in *Drosophila* revealed a useful system for the study of this phenomenon. The phenotype can be ascertained by direct observation of the adult fly.

Balanced polymorphisms involving variation in body colour have been studied in a number of species of *Drosophila*. Da Cunha (1949) analysed a colour pattern polymorphism in *Drosophila polymorpha* and showed that the variability was due to a single pair of alleles. The ratios obtained in the F2 and backcrosses indicated an excess of heterozygotes and a deficiency of homozygotes. Other studies on body colour polymorphism in *Drosophila* include those on *Drosophila montium* by Freire-Maia (1949 and 1964), on *Drosophila rufa* by Oshima (1952), on *Drosophila L. lebanonensis* by Pipkin (1962) and on *Drosophila auraria* by Lee (1963). The only analysis of a naturally occurring balanced colour polymorphism in *Drosophila melanogaster* is that done by Zurcher (1963). In all the above studies the genetic basis for the polymorphism has been shown to be a single pair of autosomal alleles.

The genetic basis of the balanced colour polymorphism under study in this thesis has been shown, by Dr. A. Robertson, to be two alleles at a locus mapping at -1 centimorgans on the third chromosome. These alleles affect the pigmentation of the sixth and seventh abdominal tergites and their effects are only detectable in females. They were first noted in lines selected for number of bristles on the sternopleural plate, the dominant allele being found in a line selected for high sternopleural bristle number and the recessive allele in a line selected downwards for this character. In an outbred population set up by Dr. A. Robertson the dominant allele at this locus was maintained at a frequency of about 40%. Further populations were set up with the dominant allele at high and low initial frequencies in order to observe the progress towards an equilibrium point and to determine whether the initial frequencies had any effect on this equilibrium point.

Some preliminary experiments indicated that the polymorphism was maintained by heterozygote superiority. In an attempt to discover the nature of this superiority, various components of fitness were examined in heterozygotes and homozygotes. Those traits selected for study were sterility, longevity, male mating ability, female fecundity, viability during two stages of development and rate of development.

Other genes affecting pigmentation of the abdominal cuticle have been located in this laboratory. The dominant allele at a locus on the fourth chromosome produced a band of pigment on the sixth tergite. The recessive allele at this locus has correlated effects on sternopleural, interocellar and abdominal bristles and reduces male and female fitness. A number of experiments involving these alleles are described.

Temperature modifies the effects of these genes. In general, a lower temperature causes an increase in the amount of pigment in the cuticle. The effect of temperature on the dominance relationships of these genes was examined and the critical period of action for these changes determined.

Experiments designed to locate other genes affecting body colour that may be present in the lines of <u>Drosophila</u> <u>melanogaster</u> maintained in this laboratory are also described.

MATERIALS AND METHODS

(i) General

All stocks were raised in half pint bottles containing standard cornmeal molasses medium in a constant temperature room maintained at 25 ± 1°C. Half-inch vials were used when it was necessary to store flies either to age them before mating or to allow the pattern of pigmentation to develop. Crosses were raised in half pint bottles except in those cases in which the number of parents was small when half-inch vials were used.

The number of parents in a bottle varied for different experiments and was fixed by results from preliminary experiments or from a knowledge of the egg laying ability of the females being used. The time the females were allowed to lay in the bottles also varied and was decided by experience to avoid too much overcrowding.

When virgin females were required, they were collected at six hourly intervals. In cases where it was necessary to use females from bottles where the last emptying had been longer than eight hours ago, only those females in which the colour had not fully developed or the gonads hardened were used.

Details of the population cages and their maintenance will be given in the Results section.

(ii) The stocks containing colour pattern genes.

The genes determining the colour patterns under study are present in stocks which have been selected for sternopleural bristle number. The Kaduna cage population from which these stocks were initially derived has been described by Clayton, Morris and Robertson (1957). The cage is still being maintained under the same conditions.

The stock, which will be referred to as DF, originated from a cross between LF_4, a line derived from Kaduna by selecting downwards to a limit of 11-4 bristles, and a low line, also derived from Kaduna, which was selected downwards for six generations. Selection at an intensity of 10/100 was carried out after seven generations of random mating following the initial cross (Osman, 1963).

DF was found to be homozygous for the recessive allele at two loci controlling the amount of pigment in the abdominal cuticle. The colour pattern characteristic of DF is a large, almost square, black spot at each side of the dorsal part of sixth tergite plus a smaller spot of pigment directly below it on the seventh tergite (cf. Figure 1).

Using this multiple recessive stock, Dr. A. Robertson located the two loci controlling the pigmentation of the abdomen. One locus was found to be on the fourth chromosome, but its precise location has not yet been determined. This requires special methods, such as the use of diplo X triploid females, to enhance crossing over on the fourth chromosome. Flies homozygous for fourth chromosomes carrying the recessive gene have reduced numbers of bristles at the sternopleural, abdominal and ocellar sites (c.f. Table 1). Male mating ability and female fertility is also reduced in these flies. Whether these are the pleiotropic effects of a single gene or are due to a complex of closely linked genes is not known. The special methods for enhancing crossing over on the fourth chromosome are necessary to determine this.

Figure 1. Photographs showing female phenotypes. From top to bottom, LF, BF and SF females.

Genotype	Number of ocellar bristles	Number of Sternopleural bristles	Number of bristles on 5th abdominal segment
β_D/β_D ; γ_R/γ_R	4.98 ± 0.15	8.73 ± 0.12	11.91 ± 0.14
β_R/β_R ; γ_D/γ_D	8.44 ± 0.07	10.44 ± 0.08	13.00 ± 0.11
β_R/β_R ; γ_R/γ_R	4.72 ± 0.12	7.84 ± 0.09	11.59 ± 0.18

TABLE 1. Number of bristles at the ocellar, sternopleural and abdominal sites of flies with the genotypes shown. The genetic background of all flies was DF. Each count is based on 100 flies, 50 of each sex.

The dominant allele at this locus on the fourth chromosome results in the production of a band of pigment across the dorsal part of the sixth tergite (c.f. Figure 1). At 25°C this allele is semidominant to that found in the DF stock. The amount of pigment in the band, and its width, in heterozygotes is very variable. In some flies so little pigment is present in the band that there is difficulty in distinguishing heterozygous females from those homozygous for the fourth chromosome recessive.

The second locus has been located on the third chromosome, to the left of the marker gene roughoid, at -1 centimorgans. Flies carrying the dominant gene at this locus show an almost complete absence of pigment on the sixth abdominal tergite (c.f. Figure 1). At 25°C this gene is epistatic to all other loci concerned with pigmentation of the sixth tergite that have been examined. It was first located in a stock C_3A, which had been selected upwards for sternopleural bristle number.

C_3A was selected from a cross between C/3 and A/5 (da Silva, 1961), both of which had reached a limit, the former at 44 bristles and the latter at 32. The reselected line reached a mean of 50 bristles.

The effects of these loci on pigmentation can only be seen in females. Those females showing an absence of pigment on the sixth tergite of the abdomen will be referred to as LF, those showing a band as BF and those with spots on either side of the sixth tergite as SF.

The dominant allele at -1 on the third chromosome will be denoted by $\underline{\beta D}$ and the recessive by $\underline{\beta R}$. The dominant allele on the fourth chromosome will be referred to as $\gamma_{\underline{D}}$ and the recessive as $\gamma_{\underline{R}}$.

The genotypes and phenotypes of females are summarised in Table 2.

All the stocks that have been examined have the dominant gene on the fourth chromosome, with two exceptions. These are the DF stock and a stock obtained from Dr. Scharloo in Holland with the sparkling cartaract gene on the fourth chromosome.

(iii) Marked stocks.

(a) Dominant markers and cross-over suppressors

The marked chromosomes used in substitution work were the following:

Cy	:	Curly of Oster	(chromosome 2)
Pm	:	Plum	(chromosome 2)
Ubx^{130}	:	Ultrabithorax of Lewis	(chromosome 3)
Sb	:	Stubble	(chromosome 3)
Xa	:	Xasta	(translocation 2; 3)
ci^{D}	:	cubitus-interruptus dominant	(chromosome 4)
ci^{W}	:	cubitus-interruptus of Wallace	(chromosome 4)

The Cy, Pm, Ubx, Sb and ci^{D} chromosomes were in a balanced stock obtained from Dr. Scharloo, Leiden University, The Netherlands.

The marker ci^{W} was not completely dominant. The mutant phenotype was found to be expressed in only 84% of heterozygotes.

Genotype	Phenotype
β_D/β_D : γ_D/γ_D	*LF*, phenotype of C_3A
β_D/β_D : γ_D/γ_R	*LF*
β_D/β_D : γ_R/γ_R	*LF*
β_D/β_R : γ_D/γ_R	*LF*
β_D/β_R : γ_R/γ_R	*LF*
β_R/β_R : γ_D/γ_D	*BF*
β_R/β_R : γ_D/γ_R	band of variable width
β_R/β_R : γ_R/γ_R	*SF*, phenotype of *DF*

TABLE 2. The phenotypes of females with the dominant and recessive genes on the third and fourth chromosomes in the combinations shown. The description of the phenotype is with respect to the pigmentation of the sixth tergite of the abdomen. The above classification only holds at 25°C.

The marker was used only in those cases in which flies with the cubitus-interruptus phenotype were being selected for the subsequent generation, as the wild type flies would be of two types, true wild type and unexpressed *ci*^{W}.

In most experiments using the above markers it was necessary that the genetic background be that of the multiple recessive stock *DF*, in order to detect differences in pigmentation. The following lines were obtained from Dr. A. Robertson.

1. *Cy* *Pm* in *DF*
2. *Ubx* *Sb* in *DF*
3. *Xa* in *DF*
4. *Xa* *ci*^{W} in *DF*
5. *ci*^{D} in *DF*
6. *ci*^{W} in *DF*

Cross-over suppressors were used to suppress crossing over in females only where absolutely necessary. Whenever possible whole chromosomes were passed through males in which no crossing over occurs. Louw (1966) tested the ability of *Cy* to suppress crossing over in females using the *alsp* chromosome described in the next section. No crossing over had occurred in the 500 flies assayed. *Ubx* was tested using the *ruca* chromosome also described in the next section. Crossing over had occurred in 0.7% of the flies assayed, but it is thought that this low frequency of crossing over does not seriously affect the results of experiments in which this cross-over suppressor was used.

(b) Other marked stocks

(1) al dp b pr c px sp

This multiply marked second chromosome is abbreviated to alsp. The stock was homozygous for the alsp chromosome. The markers are aristaless (al), dumpy (dp), black (b), purple (pr), curved (c), plexus (px) and speck (sp). The map distances given in Figure 2 are from Bridges and Brehme (1944).

(2) al dp b pr Bl c px sp / SM1

This is abbreviated to Blasp/SM1. In addition to the seven recessive markers of (1), the Blasp chromosome has the dominant mutation Bristle (Bl). The whole chromosome is balanced against SM1, which suppresses crossing over along the whole length of the chromosome.

(3) ru h th st cu sr e^s Pr ca / Ubx

This third chromosome stock is abbreviated to ruPrca/Ubx. The markers are roughoid (ru), hairy (h), thread (th), scarlet (st), curled (cu), stripe (sr), ebony/sooty (e^s), Prickly (Pr) and claret (ca). The map distances shown in Figure 2 are again from Bridges and Brehme. Prickly is a dominant, homozygous lethal mutation. The whole chromosome is balanced against the cross-over suppressor Ubx.

(4) ru h th st cu sr e^s ca

This stock is abbreviated to ruca. It is homozygous for a multiply marked third chromosome containing the eight recessive markers given in (3), but lacking the dominant marker Prickly.

2nd chromosome 'alsp'

al	dp	b	pr	c	px	sp
0.0	13.0	48.5	54.5	75.5	100.5	107.0

2nd chromosome 'Blasp'

al	dp	b	pr	BL	c	px	sp
				54.8			

3rd chromosome 'ruPr^{S}Ca'

ru	h	th	st	cu	sr	e^{s}	Pr	ca
0.0	26.5	43.2	44.0	50.0	62.0	70.7	90.0	100.7

Fig.2. Marked second and third chromosomes described in the section on marked stocks. Map distances from Bridges and Brehme (1944).

(5) *ci ey*^{R}

This stock is homozygous for a fourth chromosome containing the recessive markers cubitus-interruptus (ci) and eyeless-Russian (ey^R). In addition it is homozygous for the allele producing a band on the sixth tergite of the abdomen ($\underline{\gamma_D}$). It has been stated previously that the gene producing this band is not completely dominant and, in some cases, it is difficult to distinguish *BF* females from *SF* females. As crossing over on the fourth chromosome is a very rare occurrence, the markers, *ci* and *ey*^{R}, in this stock can be used to identify females heterozygous for the gene $\underline{\gamma_D}$. It also makes possible the identification of males, homozygous and heterozygous for $\underline{\gamma_D}$, in which the different patterns of pigmentation cannot be observed.

As with the dominant markers and cross-over suppressors, it was usually necessary to use the above marked chromosomes substituted into a *DF* background, in order to identify pigmentation patterns.

The procedures using the stocks described in this section to obtain specific genotypes will be described in the appropriate sections of the Results.

RESULTS

The effect of second and third chromosomes on pigmentation pattern

In an attempt to determine whether any genes affecting body colour, other than those mentioned in the previous section, were present in lines of *Drosophila melanogaster* maintained in this laboratory, a number of second and third chromosomes from these lines were tested for their effect on pigmentation pattern. The lines in which chromosomes were tested are shown in Tables 4 and 5.

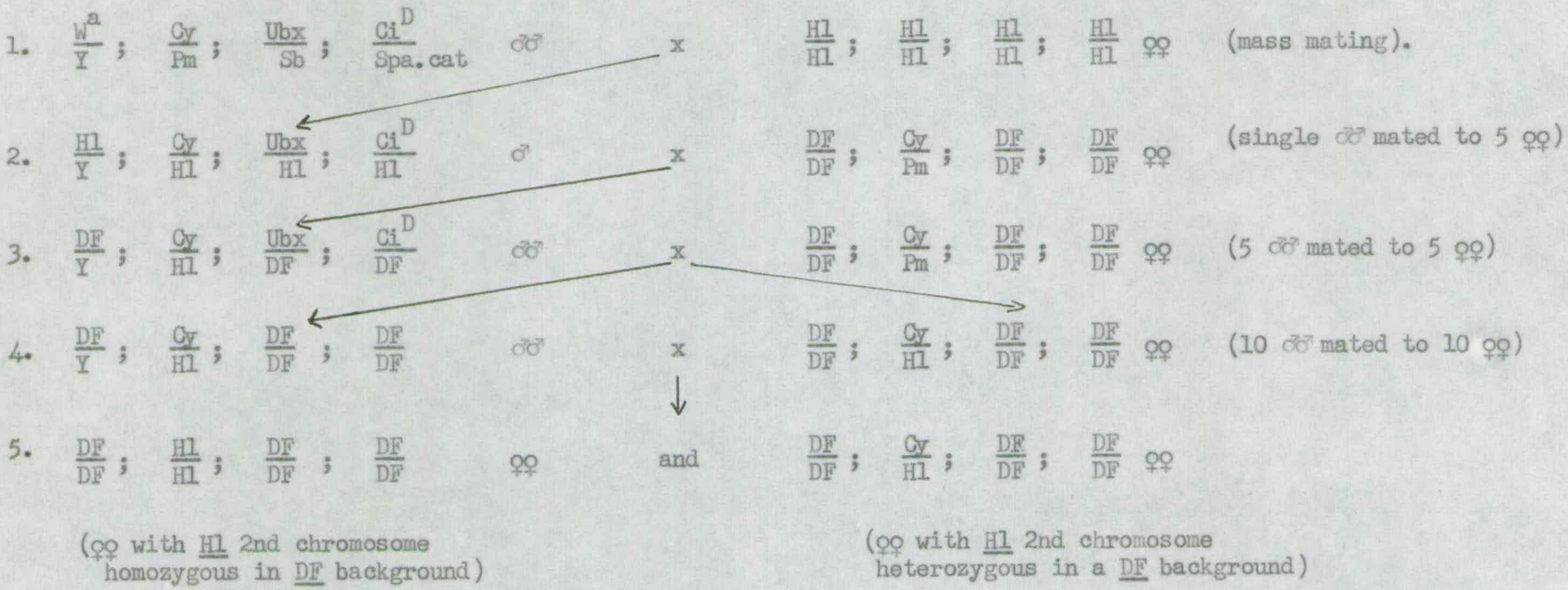
These lines were derived from the Kaduna population by selection. With one exception, B2, the character selected for was sternopleural bristle number. The three lines H1, H2 and H3 had been selected in an upward direction and the four lines MD/A, MD/B, NA/A and NA/B had been selected downwards. A selection intensity of 10/25 was applied in both high and low selected lines. At the time this study was begun, selection on these lines had been relaxed and the sternopleural bristle scores were showing a tendency to return to that of the base population. The sternopleural bristle scores for these seven lines are given in Table 3. The scores are based on counts of 100 flies, 50 of each sex.

The selection procedure used to produce line C_3A has been outlined in the previous section.

In line B2 the character selected for was width of the band of pigment on the sixth abdominal tergite. In each generation those females with the broadest band of pigment, as judged by eye, were used as parents for the next generation. No selection could be carried out on males as the expression of this character is limited

Line	Sternopleural bristle score
H1	23.74 ± 0.46
H2	22.50 ± 0.59
H3	20.98 ± 0.41
MD/A	15.34 ± 0.23
MD/B	15.78 ± 0.37
NA/A	15.62 ± 0.28
NA/B	16.24 ± 0.42

TABLE 3. Sternopleural bristle scores.



(mass mating).

(single ♂♂ mated to 5 ♀♀)

(5 ♂♂ mated to 5 ♀♀)

(10 ♂♂ mated to 10 ♀♀)

(♀♀ with Hl 2nd chromosome homozygous in DF background)

(♀♀ with Hl 2nd chromosome heterozygous in a DF background)

Figure 3. Scheme for substituting a second chromosome into a DF background. Hl is used as an example.

1. $\frac{W^a}{Y}$; $\frac{Cy}{Pm}$; $\frac{Ubx}{Sb}$; $\frac{Ci^D}{Spa.cat.}$ ♂♂ x $\frac{Hl}{Hl}$; $\frac{Hl}{Hl}$; $\frac{Hl}{Hl}$; $\frac{Hl}{Hl}$ ♀♀ (mass mating)

2 $\frac{Hl}{Y}$; $\frac{Cy}{Hl}$; $\frac{Ubx}{Hl}$; $\frac{Ci^D}{Hl}$ ♂ x $\frac{DF}{DF}$; $\frac{DF}{DF}$; $\frac{Ubx}{Sb}$; $\frac{DF}{DF}$ ♀♀ (single ♂♂ mated to 5 ♀♀)

3 $\frac{DF}{Y}$; $\frac{Cy}{DF}$; $\frac{Ubx}{Hl}$; $\frac{Ci^D}{DF}$ ♂♂ x $\frac{DF}{DF}$; $\frac{DF}{DF}$; $\frac{Ubx}{Sb}$; $\frac{DF}{DF}$ ♀♀ (5 ♂♂ mated to 5 ♀♀)

4. $\frac{DF}{Y}$; $\frac{DF}{DF}$; $\frac{Ubx}{Hl}$; $\frac{DF}{DF}$ ♂♂ x $\frac{DF}{DF}$; $\frac{DF}{DF}$; $\frac{Ubx}{Hl}$; $\frac{DF}{DF}$ ♀♀ (10 ♂♂ mated to 10 ♀♀)

5. $\frac{DF}{DF}$; $\frac{DF}{DF}$; $\frac{Hl}{Hl}$; $\frac{DF}{DF}$ ♀♀ and $\frac{DF}{DF}$; $\frac{DF}{DF}$; $\frac{Ubx}{Hl}$; $\frac{DF}{DF}$ ♀♀

(♀♀ with Hl 3rd chromosome homozygous in DF background)

(♀♀ with Hl 3rd chromosome heterozygous in DF background).

Figure 4. Scheme for substituting a third chromosome into a DF background. Hl is used as an example.

to females. The band of pigment in those females used in this study was approximately twice as broad as that found in BF females (cf. Figure 1).

As the DF stock is known to have the recessive allele at at least two loci controlling the amount of pigment in the abdominal cuticle, it was desirable to substitute chromosomes into a DF background in order to determine their effects on pigmentation pattern. The schemes used to substitute second and third chromosomes into this background are shown in Figures 3 and 4 respectively. Ten second and ten third chromosomes from each line were examined. Thus, in general, only those effects caused by genes at an appreciable frequency in the population will be observed.

In order to maintain intact chromosomes the use of females for carrying the chromosomes under study was avoided as far as possible. This precaution was particularly necessary in the case of third chromosome substitutions where the main crossover suppressor used was Ubx.

As the dominant mutations Cy and Ubx have no effect on abdominal pigmentation, it was possible to determine whether the effect due to a certain chromosome was dominant or recessive by examining Cy or Ubx females from generation 5 of the substitution procedure.

The pigmentation patterns observed on the sixth abdominal tergite, when second chromosomes from the various lines were substituted into a DF background, are shown in Table 4. In only two lines, Hl and NA/A, was any observable effect on pigmentation

Line	Effect of substituted second chromosomes when homozygous	
	Number	Phenotype
Kaduna	10	SF
H1	9 1	SF spots smaller
H2	10	SF
H3	9 1	SF lethal
MD/A	10	SF
MD/B	10	SF
NA/A	4 2 4	SF lethal spots smaller
NA/B	9 1	SF lethal
C_3A	10	SF
B2	10	SF

TABLE 4. The effect of homozygous second chromosomes on the pigmentation pattern of the sixth abdominal tergite.

found. In these two lines the spots characteristic of the SF phenotype were smaller and less densely pigmented. If the small sample of second chromosomes examined can be regarded as representative of the population from which they were taken, the factor causing suppression of the spots is at a lower frequency in Hl, only 10% of second chromosomes carrying it, than in NA/A where 40% carried it. In Hl this factor may be at a low frequency and only have been picked up by chance, but in NA/A it appears to be at an appreciable frequency.

Cy females showed the SF phenotype indicating that the effect was recessive.

The degree of suppression of the amount of pigment in the sixth tergite was variable. Females homozygous for a single second chromosome showed a range of phenotypes from almost complete suppression to almost complete expression of the spots.

An attempt was made to determine the precise location of the factor on the second chromosome using Blasp and alsp chromosomes substituted into a DF background. The method was essentially the same as that used by Louw (1966) to locate the chromosomal regions affecting sternopleural bristle number in lines selected upwards and downwards for this trait. The effect was found to be too variable to make precise location possible.

The effects of third chromosomes from the various lines on pigmentation pattern of the sixth tergite are shown in Table 5. Two types of effect were observed.

Line	Effect of substituted third chromosomes when homozygous	
	Number	Phenotype
Kaduna	8	SF
	1	lethal
	1	FB
H1	7	SF
	2	LF
	1	FB
H2	6	SF
	2	LF
	1	lethal
	1	FB
H3	6	SF
	3	LF
	1	FB
MD/A	6	SF
	3	LF
	1	FB
MD/B	7	SF
	3	LF
NA/A	6	SF
	1	LF
	1	lethal
	2	FB
NA/B	8	SF
	2	LF
B2	9	SF
	1	FB

TABLE 5. The effect of homozygous third chromosomes on the pigmentation pattern of the 6th abdominal tergite.

All the stocks tested, with the exception of *Kaduna* and *B2*, contained third chromosomes carrying the gene $\underline{\beta_D}$, which produces the *LF* phenotype. The results of a small experiment, in which the seven lines selected for sternopleural bristle number were crossed to *DF*, had indicated that these lines contained $\underline{\beta_D}$. The proportions of *LF* females found in the various lines, on crossing with *DF*, are shown in Table 6.

The results of the substitution of third chromosomes from the *Kaduna* population into *DF* were unexpected. In the next section it will be shown that, in populations in which the two alleles at the $\underline{\beta}$ locus were segregating in a *DF* background, an equilibrium point was reached at which the frequency of $\underline{\beta_D}$ was about 0.50. In view of this, it might be expected that the allele would be at an appreciable frequency in the *Kaduna* population. Experiments by Dr. A. Robertson and Dr. C. McPhee indicated that the frequency of $\underline{\beta_D}$ in this population was about 0.50. The reason why chromosomes containing this allele were not present in this sample is not clear. In view of the small number of chromosomes examined, one possibility is that it could be due to the sampling procedures. Sampling was involved at two stages in the substitution procedure. Females used to initiate the substitution procedure were obtained by sampling the *Kaduna* population. Eggs samples were used and the eggs in these will not represent all the females in the cage. In the second generation of the substitution procedure, further sampling was involved as ten single chromosomes were selected from the population.

Line	% LF
H1	8.5
H2	5.5
H3	9.6
MD/A	28.2
MD/B	23.7
NA/A	2.3
NA/B	7.3

TABLE 6. Proportion of LF females in each line on crossing with DF.

The second type of effect was observed in all the lines tested, with the exception of MD/B and NA/B. The sixth tergite had a faint band of pigment across it and the spots were smaller than those in SF. This phenotype will be referred to as FB. The effect is recessive, Ubx females showing the SF phenotype.

Experiments by Dr. A. Robertson indicate that the effect may be due to a gene at, or closely linked to, the ebony locus. Females from C_3A were backcrossed to a line containing the mutant ebony sooty (e^s) in a DF background and selected downwards for sternopleural bristle number in each generation. When the sternopleural bristle score reached 10, non e^s females were crossed to Ubx/ruPrca males. Ubx, non ebony males and females were selected from this cross and mated. The pigmentation pattern on the sixth tergite of wild type females emerging from this cross was observed. It was similar to the FB phenotype described above.

This finding was confirmed when C_3A females were backcrossed to e^s in DF males for 18 generations. Eight replicates were set up, each being initiated from a single C_3A female. After 18 generations of backcrossing, non e^s flies will be heterozygous for a small region of the C_3A third chromosome including the ebony locus, the rest of the genotype being that of the e^s in DF line. This was confirmed by sternopleural bristle counts. The amount of C_3A third chromosome present will vary between replicates, depending on the number of cross overs that have occurred in the region of the ebony locus in each. This portion of C_3A third chromosome was made homozygous in each replicate. To do this, non e^s females from the eighteenth generation were crossed to

Ubx/ru Pr ca in DF males. Ubx, non ebony flies were selected and crossed, and the wild type females emerging from this cross will be homozygous for this small region of the C_3A third chromosome. The pigmentation pattern on the sixth abdominal tergite of these wild type females was examined. Of the eight replicates set up, females from three showed the FB phenotype. In the other five, no faint band was present on the sixth tergite, although the spots on this tergite tended to be smaller than those in SF.

These results can be interpreted in two ways. Only one locus may be involved, either the ebony locus or one closely linked to it, the effect of this locus on pigmentation of the sixth tergite being a variable one. Alternatively two closely linked loci may be involved, one of which may be the ebony locus. On this hypothesis, a cross over must have occurred between these two loci in five of the replicate lines, so that the locus responsible for the production of the faint band of pigment was no longer present in the small region of C_3A third chromosome retained in these lines.

In B2, the line selected for width of band, the effect of second and third chromosomes on banding was not noticeably greater than in a number of other lines not selected for this trait. If modifiers are responsible for the increase in the width of the band, they must be located on the X or fourth chromosomes.

A number of the chromosomes examined were found to be lethal when homozygous. The lethal frequency among second chromosomes was 0.04 and among third chromosomes 0.03. Two lethal second chromosomes were extracted from NA/A, but these may be identical.

Studies on a body colour polymorphism

The body colour polymorphism under study has been shown to be controlled by a single pair of alleles, $\underline{p_D}$ and $\underline{p_R}$, located at -1 centimorgans on the third chromosome. In the experiments described in this section the alleles $\underline{p_D}$ and $\underline{p_R}$ are in a *DF* background. A uniform genetic background is necessary in order to show that the characteristics of the polymorphism are due only to this single pair of alleles and not to other genes that may be present. A *DF* background is used because *DF* is the only stock in this laboratory with the recessive allele at the fourth chromosome locus controlling abdominal pigmentation. The presence of the dominant allele at this locus would mask the expression of $\underline{p_R}$.

1. Cage and bottle population studies

In a cage population, set up by Dr. A. Robertson, with $\underline{p_D}$ and $\underline{p_R}$ segregating in a *DF* background, an equilibrium point was reached with $\underline{p_D}$ at a frequency of about 0.40. To confirm this finding and to determine if initial gene frequency in any way affected the final equilibrium point, two bottle populations were set up. In one the initial frequency of $\underline{p_D}$ was 0.75. After ten generations the bottle population was split, half being maintained in bottles and half transferred to a cage. In the second population $\underline{p_D}$ was at an initial frequency of 0.25 and this population was split in the same manner after six generations in bottles.

It was decided to examine the approach to equilibrium in both cage and bottle populations because the two types of population differ in a number of aspects. The generations in a bottle population are discrete, while those in a cage population are overlapping, and this may have some effect on the final equilibrium frequency. Factors such as longevity and rate of development are likely to play a more important role in populations with overlapping generations. A degree of overcrowding was maintained in the bottle populations, but it is probable that this was not as great as that in the cage populations. Thus competition would be more severe in the latter. The conditions in cage populations approximate rather more to natural conditions than do those in bottle populations, but the latter have the advantage that the change in gene frequency can be measured in terms of generations rather than in terms of weeks.

a. Maintenance of the populations

i. Bottle populations

Each bottle population consisted of five bottles. In each generation the flies from each bottle were scored over a period of eight days following the first emergence. These flies were then used as parents for the next generation and were equally divided between another five bottles. The females were allowed to lay for a length of time determined by experience, such that there was some degree of overcrowding.

ii. Cage populations

The population cages used were similar to that used for the Kaduna population described by Clayton, Morris and Robertson (1957). The cage is a wooden box with a glass top, with outside dimensions 13 x 13 x 10 inches. Three food jars, containing cornmeal-molasses-agar culture medium to which a little live yeast has been added, are placed at one side of the cage. The oldest food jar is removed each week, so that a food jar remains in the cage for three weeks. In one side of the cage is a circular opening covered by fine gauze. Through this flies can be introduced and withdrawn, and the food jars exchanged.

One of the problems in experiments using population cages is that it is impossible to control any mite infection which may develop in the cage. If mites appear, the experiment must be terminated. The cages were checked at intervals for possible mite infection.

Another problem is the possibility of contamination by *Drosophila* from outside sources. As a check that no such contamination had occurred, periodic sternopleural bristle counts of females homozygous for $\underline{\rho_R}$ were made. Flies with this genotype have a lower sternopleural bristle score than any of the other stocks maintained in this laboratory. Thus contamination would be indicated if the sternopleural bristle scores of these females were significantly greater than expected.

The frequencies of $\underline{\rho_D}$ and $\underline{\rho_R}$ in the experimental populations were determined at three-weekly intervals. Egg sampling was decided upon in preference to withdrawing and replacing adults in the cages, as the risk of contamination is lower. To sample, two bottles were placed in each cage and the females in the population allowed to lay in these. Overcrowding was avoided to keep competition during the larval and pupal stages at a minimum. The bottles were removed from the cages and placed at 25 ± 1°C. When the progeny began to emerge, counts were made at daily intervals for eight days.

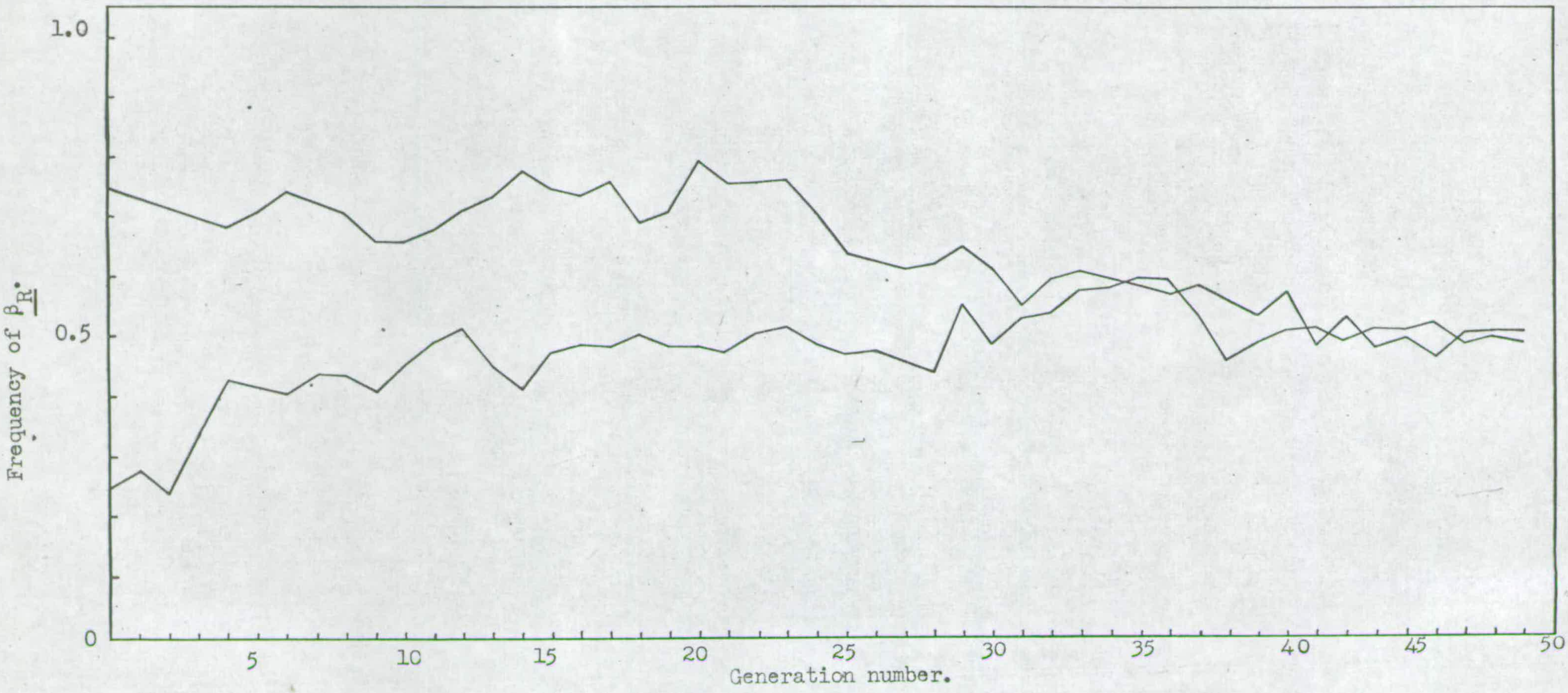
Two main sources of error are apparent in this method of sampling. Flies of different genotypes may have preferences for different parts of the cage, in which case the bottles may give a biased sample. A preliminary experiment in which vials were placed at many different points in the cage, and the progeny obtained from these egg samples examined, indicated that no such preference existed.

A second source of error derives from the fact that the eggs deposited in the sample bottles do not come from all the females present in the cage. If females of one genotype are less fit than those of other genotypes present in the cage, these females may be less able to find suitable egg laying sites in the food jars and will be more likely to lay eggs on the fresh food in the sample bottles. However, results indicate that this is not a significant source of error.

b. Results

The change in gene frequency of $\underline{\beta_R}$ per generation in population bottles is shown in Figure 5, for populations begun with high (0.75) and low (0.25) frequencies of $\underline{\beta_D}$. In the former, the frequency of $\underline{\beta_R}$ increased, while, in the latter, it decreased. After 34 generations, the frequency of $\underline{\beta_R}$ was approximately the same in the two populations. In generation 38 a drop in the frequency of $\underline{\beta_R}$ occurred in both populations, this being much sharper in the population begun with $\underline{\beta_D}$ at a high frequency. A check on the bristle scores of SF females showed that contamination was not responsible for this drop in frequency. It is possible that it was due to a change in the condition within

Figure 5. The change in the frequency of $\underline{\beta_R}$ per generation in bottle populations begun at high and low frequencies of $\underline{\beta_D}$. The upper line represents the population in which the initial frequency of $\underline{\beta_D}$ was low (0.25) and the lower line that in which the initial frequency of $\underline{\beta_D}$ was high (0.75)



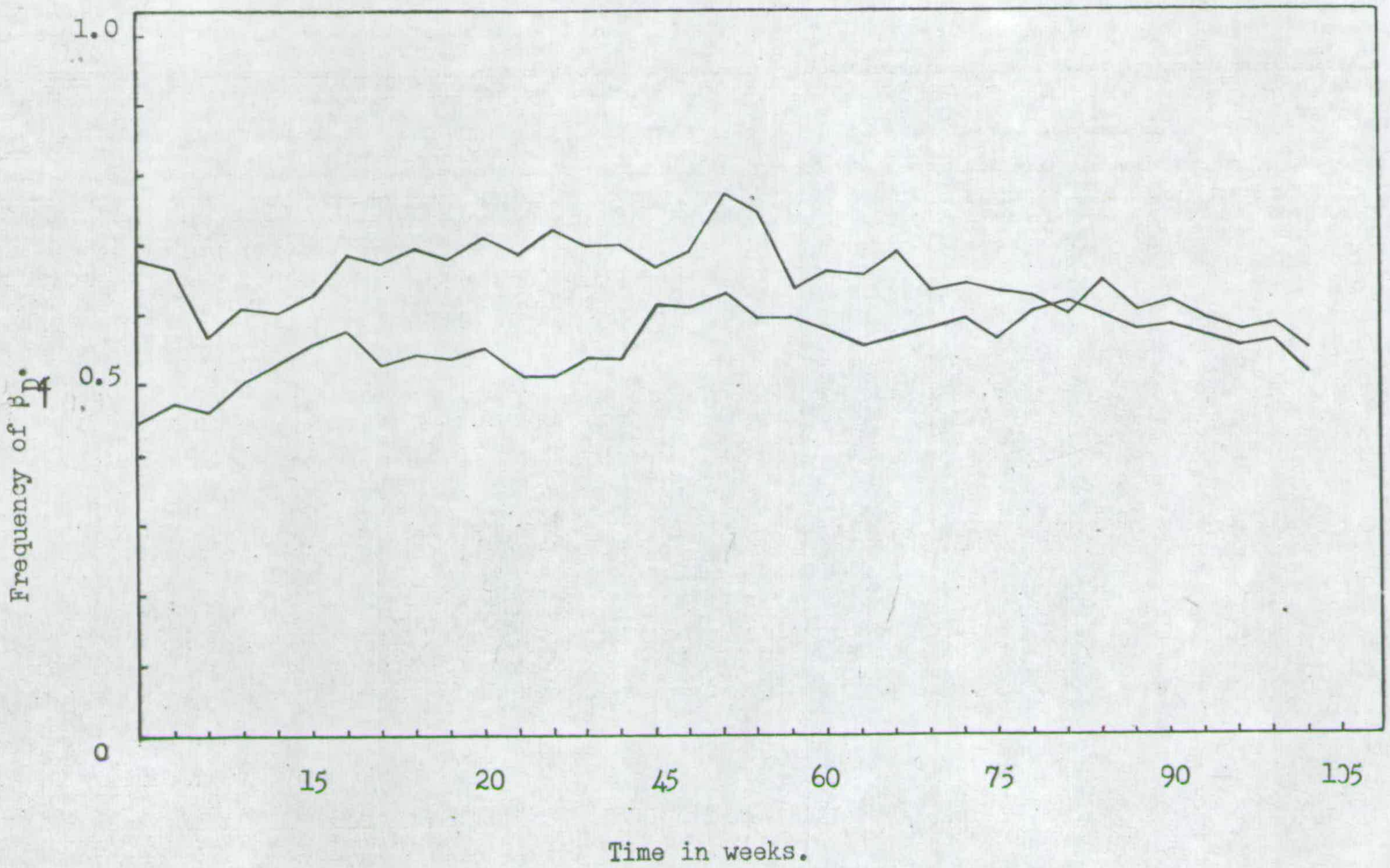
the bottles, although the precise nature of this change was not clear. By generation 41 the frequency of β_R was again the same in both populations. This frequency, which was approximately 0.5, had been maintained for eight generations when the bottle populations were terminated and probably represents the equilibrium frequency in these populations.

The change in the frequency of β_R in terms of weeks is shown in Figure 6 for the two cage populations begun at high and low frequencies of β_D. After 81 weeks the frequency of β_R was approximately the same in both populations. Although the two populations have the same frequency of β_R at this point, this frequency does not appear to represent the equilibrium frequency, as a slight downward trend was apparent and was still continuing after 102 weeks, when the sampling was discontinued.

As generations overlap in cage populations, a direct estimate of the generation interval was not possible. Under the more rigorous conditions found in cage populations, the length of a generation is likely to be longer than in bottle populations. Barker's estimate (1962) of 23 days was used as the generation interval. Thus, at the time when sampling ceased, 34 generations had elapsed in the cage populations.

After 48 generations in population bottles and 34 in population cages, β_D and β_R were still being maintained at intermediate frequencies, indicating that this pair of alleles constitutes a balanced polymorphism. The fact that populations, begun with high and low frequencies of β_D, reached the same frequency of β_R indicated that the initial gene frequency did not affect the final equilibrium frequency.

Figure 6. The change in the frequency of β_R in terms of weeks in cage populations [illegible] and low frequencies of β_D. The upper line represents the population in which the initial frequency of β_D was low and the lower line that in which the initial frequency of β_D was high.



2. Randomness of mating

A balanced polymorphism may be maintained if some type of preferential mating occurs in the population. Rendel (1951) showed that, in *Drosophila melanogaster*, ebony and vestigial males both tend to mate preferentially with females of the opposite genotype. A similar situation has been shown to exist in populations of *Panaxia dominula* (Sheppard, 1952). He demonstrated that females of *dominula*, *medionigra* and *bimacula* all prefer to mate with males of a different genotype to their own. It was therefore of importance to determine whether such a system of preferential mating was responsible for the maintenance of the polymorphism under discussion here.

Experiments using a multiple choice technique were carried out. Ten males of a given genotype, aged for three days, were placed in bottles with ten females, also aged for three days, of each of the three genotypes shown in Table 7. After three hours the females were separated from the males and each female tested for the production of progeny. As $\underline{\rho_D}$ is dominant to $\underline{\rho_R}$, it was not possible to distinguish between females homozygous and heterozygous for $\underline{\rho_D}$ on their removal from the test bottle. To do this it was necessary to examine the progeny of these females. When the genotype of the males in the cross was ρ_D/ρ_D, it was necessary to further test the progeny of ρ_D/ρ_D and ρ_D/ρ_C females by crossing to *DF* in order to determine the genotype of the female parent.

The results are shown in Table 7. No significant deviations from randomness of mating were observed.

Males	% fertilised females		
	β_D/β_D	β_D/β_R	β_R/β_R
β_D/β_D	22.0	25.0	19.0
β_D/β_R	25.0	27.0	17.0
β_R/β_R	20.0	24.0	21.0

TABLE 7. Percentages of fertile females of the three types obtained in cultures in which these females were exposed to insemination by males of one of these types. 100 females of each type were tested with each type of male.

3. Fitness studies

The data in Tables 8 and 9 indicated an excess of heterozygotes in these crosses. In the previous section it has been shown that this excess is not due to selective mating. A possible explanation is that the heterozygote is superior in fitness to either homozygote. In this case genes are expected to be maintained in the population at intermediate frequencies.

If two alleles, A and a, have gene frequencies of p and q respectively and the heterozygote, Aa, is assumed to have a selective value of 1, while the homozygotes have selective values of 1 - s and 1 - t, then the change in frequency of gene a in one generation, denoted by Δq, is given by the expression

$$q = \frac{pq\,(sp - tq)}{1 - sp^2 - tq^2} \qquad (1)$$

At equilibrium $\Delta q = 0$ and, at this point, $sp = tq$. The relative gene frequencies at the equilibrium point are therefore

$$\frac{p}{q} = \frac{t}{s} \quad (2) \quad \text{i.e.} \quad q = \frac{s}{s + t} \quad (3).$$

Thus, if the gene frequency has any value, except 0 or 1, selection will change it towards the intermediate equilibrium point, given in equation (3) and both alleles will be maintained in the population. From equation (3) it can be seen that the equilibrium gene frequency is determined by the relative disadvantages of the two homozygotes.

There are two ways in which heterozygote superiority can be demonstrated, from a study of the expected consequences of this superiority in populations or from a direct comparison of heterozygotes and homozygotes. The data from the bottle and cage populations are consistent with the hypothesis of heterozygote

<u>LF</u> females		<u>SF</u> females	
Number Observed	Number Expected	Number Observed	Number Expected
762	703.5	645	703.5

TABLE 8. Numbers of <u>LF</u> (heterozygous) and <u>SF</u> (homozygous) females emerging from cross β_D/β_R males x β_R/β_R females.

<u>LF</u> females		<u>SF</u> females	
Number observed	Number Expected	Number Observed	Number Expected
431	393	355	393

TABLE 9. Numbers of <u>LF</u> (heterozygous) and <u>SF</u> (homozygous) females emerging from cross β_R/β_R males x β_D/β_R females.

superiority. The two alleles, $\underline{\beta_D}$ and $\underline{\beta_R}$ are maintained at approximately equal frequencies, indicating that the selective values of the two homozygotes must be of the same order of magnitude.

A direct comparison of heterozygotes and homozygotes has been made in comparatively few cases. In this study, comparisons of the four possible karyotypes, β_D/β_D, β_D/β_R, β_R/β_D and β_R/β_R were made, with respect to the various components of total fitness listed below.

(a) Proportion of sterile matings
(b) Competitive mating ability of males
(c) Preadult viability
(d) Rate of development
(e) Fecundity of females
(f) Longevity

The two types of heterozygote, β_D/β_R, representing heterozygotes from matings in which the genotype of the female parent was β_R/β_R, and β_R/β_D, representing those in which it was β_D/β_D, were included to determine if any type of maternal effect was acting.

(a) Sterility

Sterility is here defined as that proportion of single pair matings which produce no progeny and was determined for the pairwise combinations shown in Table 10 . In each case two hundred single pair matings were set up in vials. The males and virgin females used were stored separately in vials for three days before being mated. After three days the flies were

Cross ($\male$ x $\female$)	% Sterile matings
β_D/β_D x β_D/β_D	10.0
β_D/β_R x β_D/β_R	8.5
β_R/β_D x β_R/β_D	8. 0
β_R/β_R x β_R/β_R	12.5
β_D/β_D x β_R/β_R	11.5
β_D/β_R x β_R/β_R	9.5
β_R/β_D x β_R/β_R	8.0
β_R/β_R x β_D/β_D	10.5
β_R/β_R x β_D/β_R	9.0
β_R/β_R x β_R/β_D	9.0

TABLE 10. Proportion of sterile crosses.

transferred, without etherisation, to another set of vials. Two further transfers were made at three day intervals. Only those vials in which both flies remained alive after the three transfers were scored. The vials were kept at 25 ± 1°C for twelve days after removal of the parent flies. If no progeny emerged from a single pair mating, that mating was regarded as sterile.

The percentage sterility for each pairwise combination is shown in Table 10. No significant differences in sterility were found. An appreciable number of sterile matings were found in all crosses and studies on the fourth chromosome locus with an effect on pigmentation indicated that this may be due to all flies used in this study being homozygous for the recessive allele at this locus.

(b) Competitive mating ability of males

The measurement of male fertility presents a problem. A large difference in total sperm production may not be reflected in the total number of progeny produced. One component of male fertility is mating ability, which may be defined as the number of females fertilised by a male in a given time, or as the success of a male in obtaining a mate when in competition with other males. Hiraizumi and Crow (1960) measured the former, but this ignores the element of competition for mates which exists in populations. In this study the component of male fertility measured is competitive mating ability. This was done in the following way.

Two males of different genotypes were allowed to compete for a single female. Examination of the progeny of this female would determine the male parent. The females used were from the DF line, which is homozygous for the recessive allele at the p locus, thus enabling the genotypes of the progeny to be determined without further testing. It was possible to detect double matings when males homozygous for $\underline{\beta_D}$ were competed with those homozygous for $\underline{\beta_R}$, because the progeny from single vials would be of two types instead of a single type expected if the female had mated with only one male. The genotypes of the pairs of competing males are shown in Table 11.

The two males, 3 days old, were placed in a vial with a DF female, of the same age, for 12 hours. The males were then removed and the female allowed to lay for 6 days. The progeny were scored. The results are shown in Table 11. No significant differences were found.

(c) Preadult viability.

Preadult viability was determined for two intervals of development, that from the first larval instar stage until emergence and from the egg stage until emergence. Viability is defined as the proportion of adults hatching from a given number of larvae or eggs. From the viabilities during these two stages of development an estimate of egg hatchability can be obtained.

i. Viability from first larval instar stage to emergence.

Crowding may have a differential effect on the four genotypes under study. Lewontin (1955) showed that density of larvae per vial had an effect on the number of adults hatching from a given number of larvae. The optimum viability occurred at intermediate densities.

Preliminary experiments were undertaken to determine the number of larvae per vial from which the optimum proportion of adults hatched. Vials were set up with 2, 10, 20, 30, 50, 100 and

Genotype of male parent				
β_D/β_D	β_D/β_R	β_R/β_D	β_R/β_R	β_D/β_D and β_R/β_R
98	102			
92		108		
105			88	7*
	94		106	
		111	89	

TABLE 11. Competitive mating ability of males.

* the only cross in which double matings occurred.

250 first instar larvae. The method of obtaining the larvae and transferring them to vials is outlined below.

Mass matings were set up to produce the required types of larvae. After three days the males were removed and the fertilised females allowed to age for a further day. These females were then transferred to new bottles containing large amounts of yeast suspension. This 'feeding up' of the females stimulates egg laying. After twenty four hours the females were transferred to bottles containing no food. These bottles were inverted over 2" watch glasses containing 10 ml. cornmeal-molasses-agar food plus a small amount of yeast suspension. A strip of absorbent paper was also introduced into each bottle to prevent the sides of the bottle from becoming too moist.

In Drosophila melanogaster it has been shown that maturation and a variable amount of embryonic development may occur while the egg is still in the uterus. 'Feeding up' the females stimulates them to lay and so the first eggs laid after this treatment will be at various stages of development. To ensure that eggs used are at approximately the same developmental stage, those laid during the first 24 hours after 'feeding up' were discarded. New watch glasses were substituted for those removed and females allowed to lay for 2 hours before removal. After 20 hours at 25 ± 1°C the majority of eggs had hatched and the first instar larvae were removed to vials using a metal 'spoon'. Using a line in which larval viability was high, it was demonstrated that this treatment did not injure the larvae. The number of adults emerging from the vials was counted every second day after the first emergence.

Graphs showing the number of adults hatching at various densities are shown in Figures 7, 8, 9 and 10. The optimum viability occurred at a density of 30 larvae per vial in each case. At lower densities there was a slight decrease in viability, possibly due to souring of the food. A significant decrease in viability was observed at higher densities which probably approximate more nearly to the situation in population bottles and cages.

Table 12 shows the number of adults hatching from 30 larvae of each genotype. Each result was based on the number of adults hatching from 30 vials. No significant differences were observed, although the number of adults hatching from β_R/β_D larvae did tend to be slightly higher than in the other three genotypes.

ii. Viability from egg stage to emergence

As no significant decrease in number of adults hatching at densities lower than 30 larvae per vial was observed, it was decided to use a density of 30 eggs per vial. The method of collecting eggs was essentially the same as that used to collect larvae, except that eggs were transferred from the watch glasses to vials immediately after the removal of females from the bottles. The number of adults emerging was again recorded every second day after the first emergence.

Table 13 shows the number of adults hatching from 30 eggs of each genotype. Each result represents the mean number from 30 vials. The viability during this part of the life cycle was significantly higher in heterozygotes than in homozygotes, no differences being apparent between homozygotes or between heterozygotes. Thus some type of heterozygote superiority existed in this trait.

A rough estimate of egg hatchability could be made using

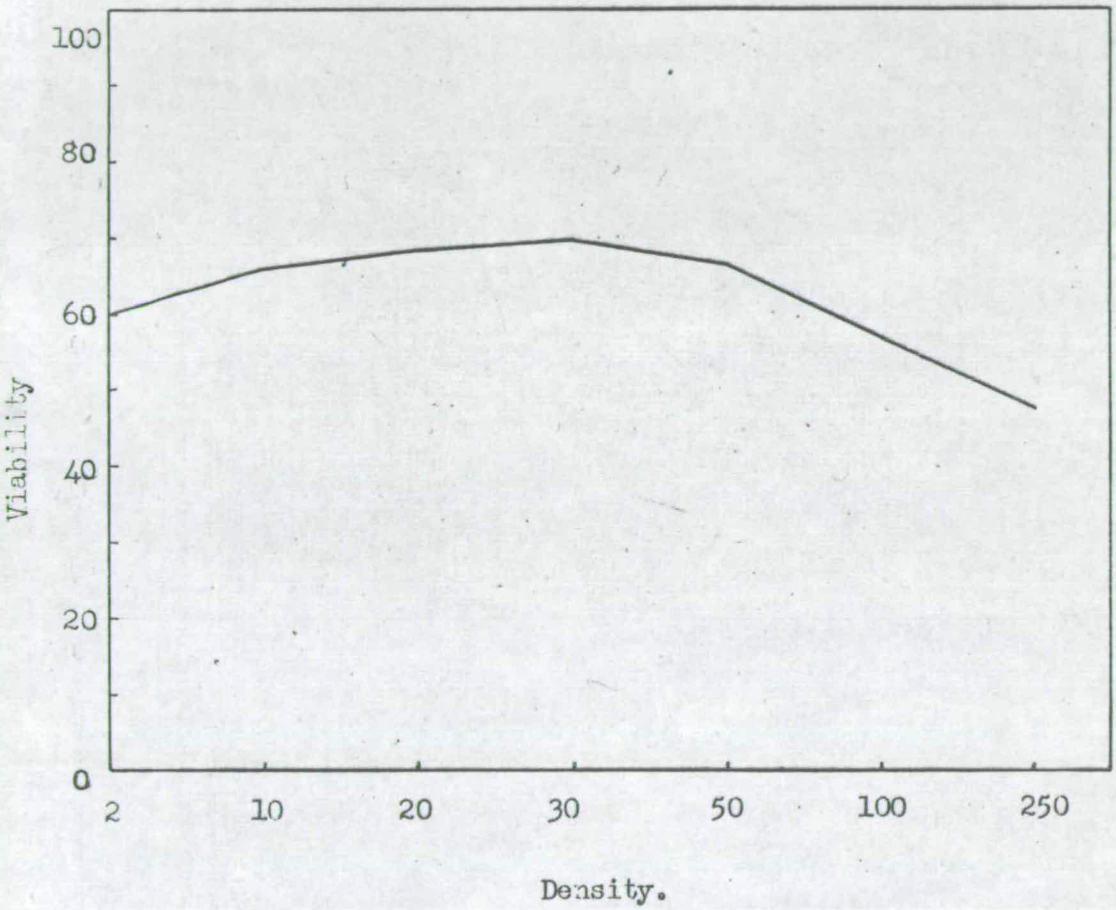


Figure 7. The viability of β_D/β_D flies at various densities of larvae per vial. Viability is defined as the number of adults hatching from 10 vials as the proportion of the number of larvae placed in those vials.

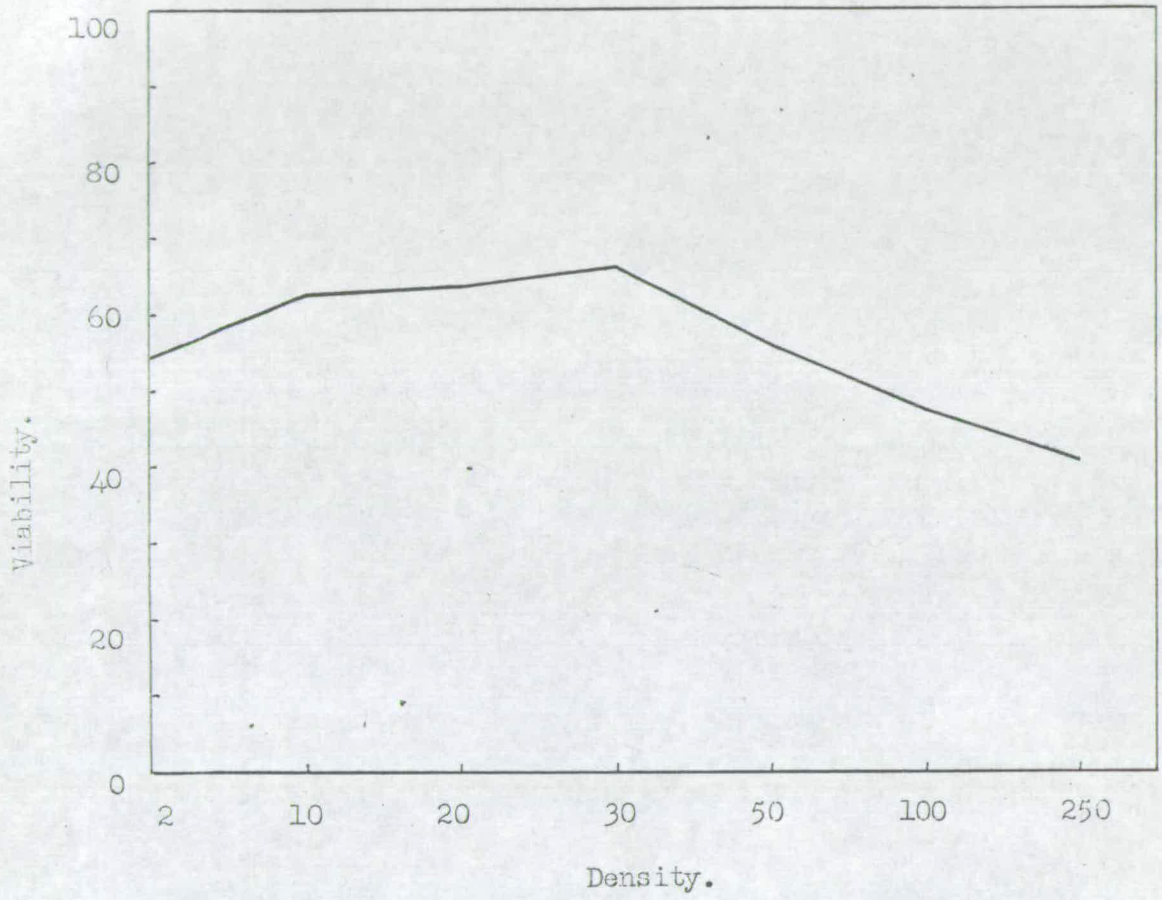


Figure 8. The viability of β_D/β_R flies at various densities of larvae per vial. Viability is defined as the number of adults hatching from 10 vials as a proportion of the number of larvae placed in those vials.

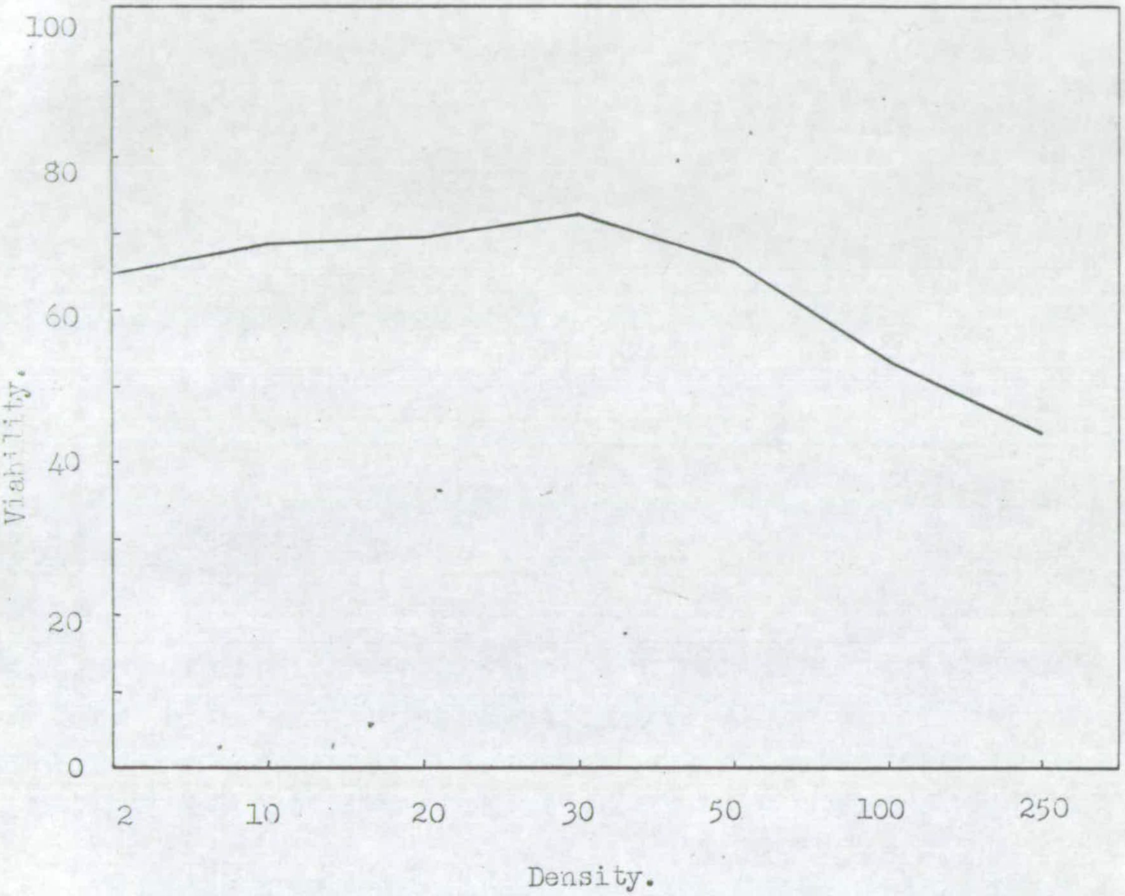


Figure 9. The viability of β_R/β_D flies at various densities of larvae per vial. Viability is defined as the number of adults hatching from 10 vials as a proportion of the number of larvae placed in those vials.

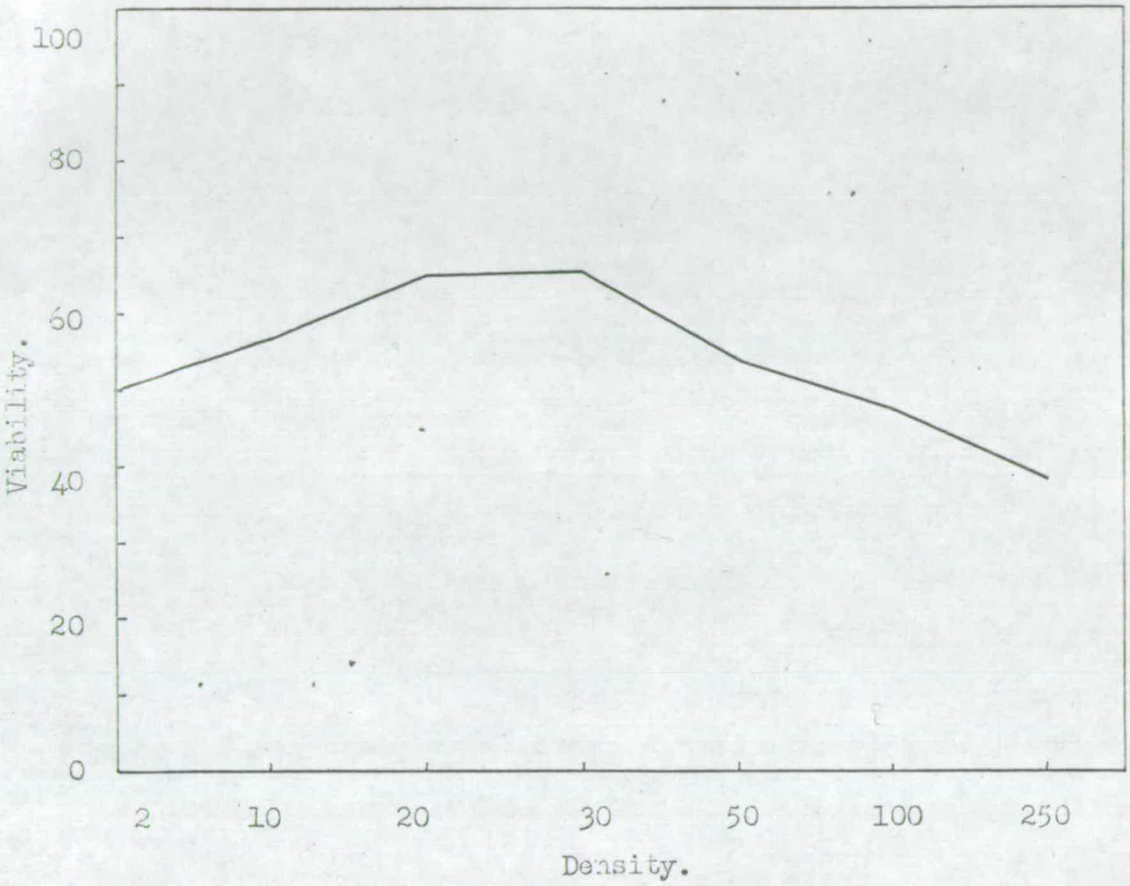


Figure 10. The viability of β_R/β_R flies at various densities of larvae per vial. Viability is defined as the number of adults hatching from 10 vials as a proportion of the number of larvae placed in those vials.

Genotype			
β_D/β_D	β_D/β_R	β_R/β_D	β_R/β_R
21.77 ± 0.43	20.33 ± 0.52	20.93 ± 0.33	21.43 ± 0.61
21.30 ± 0.76	20.77 ± 0.71	23.47 ± 0.93	18.80 ± 1.17
19.63 ± 0.81	19.40 ± 0.92	22.73 ± 0.65	20.13 ± 0.93

TABLE 12. Viability from first larval instar stage to eclosion. The number of adults hatching from 30 larvae.

Genotype			
β_D/β_D	β_D/β_R	β_R/β_D	β_R/β_R
8.87 ± 0.97	16.13 ± 0.73	15.50 ± 0.81	10.87 ± 1.25
10.30 ± 1.03	13.73 ± 0.92	16.73 ± 0.42	7.70 ± 1.07
11.90 ± 0.79	12.47 ± 0.84	15.16 ± 0.78	11.43 ± 1.32

TABLE 13. Viability from egg stage to eclosion. The number of adults hatching from 30 eggs.

the data in Tables 12 and 13. The mean of the three results in each table was calculated for the four genotypes and the viability from the egg stage to eclosion divided by that from first larval instar stage to eclosion. These estimates of egg hatchability are shown in Table 14. Hatchability was about 70 per cent in heterozygotes and about 50 per cent in homozygotes. It is probable that this is the stage in development at which the heterozygote superiority, mentioned above, was manifested as it was not apparent during the stages from first larval instar to eclosion.

(d) Rate of development

The rate of development from the first larval instar stage to eclosion was determined for each of the four genotypes. The method of collecting larvae has been described in the previous section. A density of 30 larvae per vial was used. The number of adults emerging was counted every four hours and the results are plotted in Figures 11, 12, 13 and 14., as the proportion of the total emergence. Each result represents the mean emergence,at a particular time,from 30 vials. The measurement of rate of development used was the mean length of time from first larval instar to eclosion and this is shown, for each genotype, in Table 15. β_R/β_R individuals had a significantly slower rate of development than the other genotypes examined. On average, the developmental rate of β_R/β_R individuals was about two per cent slower.

(e) Female fecundity

The number of eggs laid by fertile females of the four

Trait	Genotype			
	β_D/β_D	β_D/β_R	β_R/β_D	β_R/β_R
Viability from egg stage to eclosion	10.36	14.08	15.80	10.00
Viability from first larval instar stage to eclosion	20.90	20.17	22.38	20.12
Egg hatchability	0.50	0.70	0.71	0.50

TABLE 14. Estimates of egg hatchability.

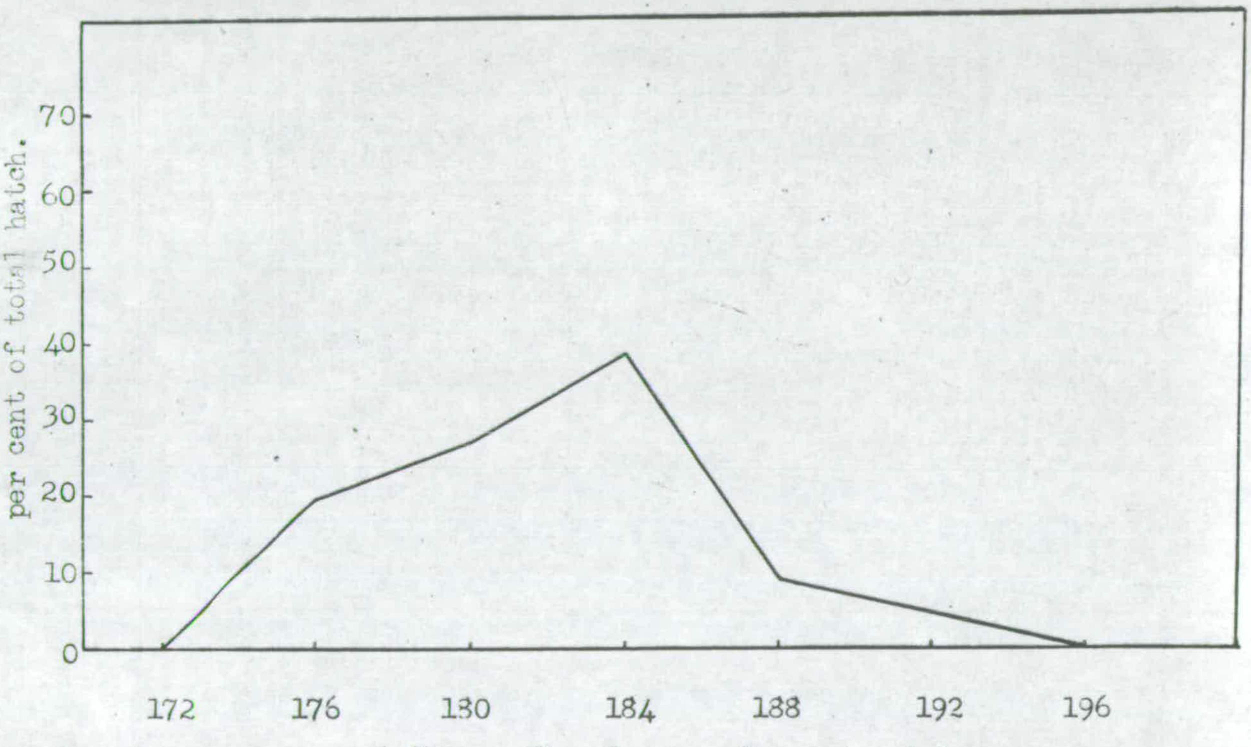


Figure 11. Age distribution of emergence of β_D/β_D flies.

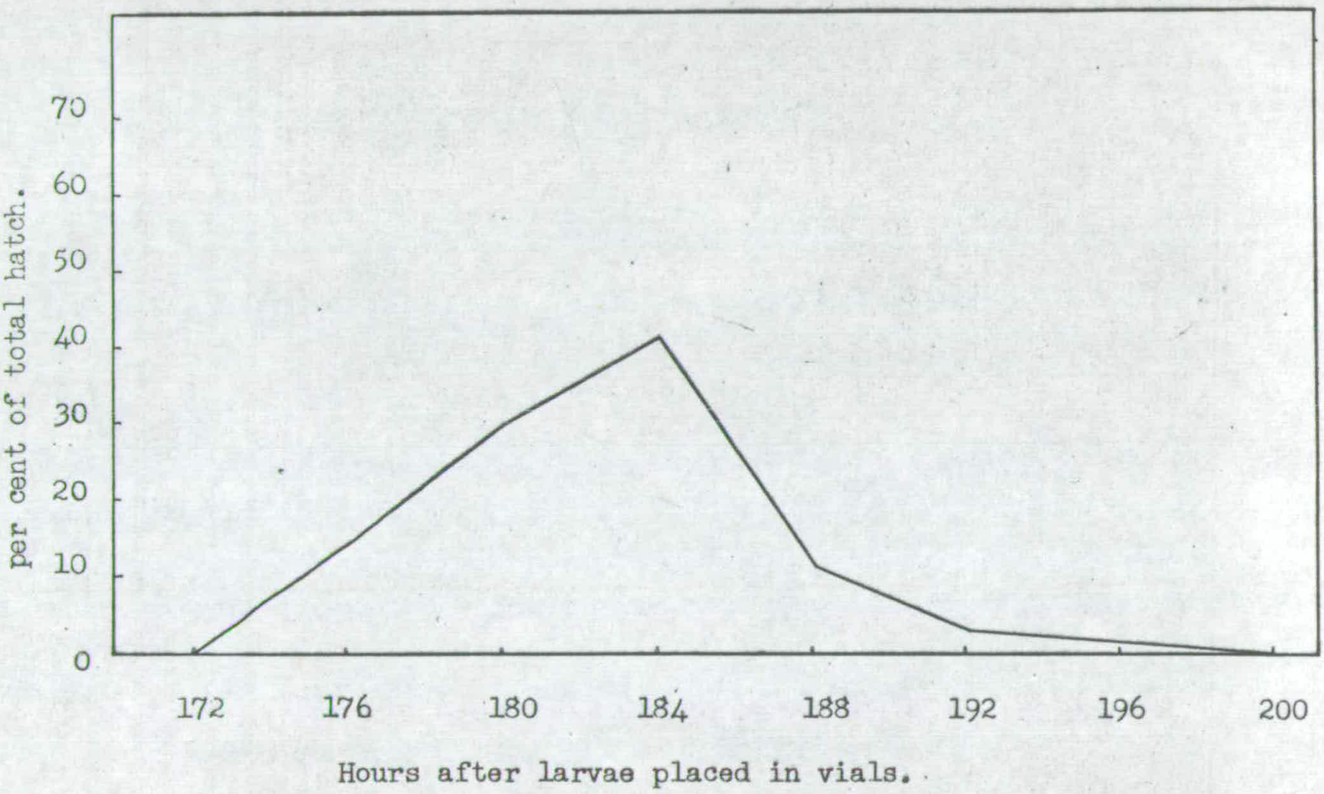


Figure 12. Age distribution of emergence of β_D/β_R flies.

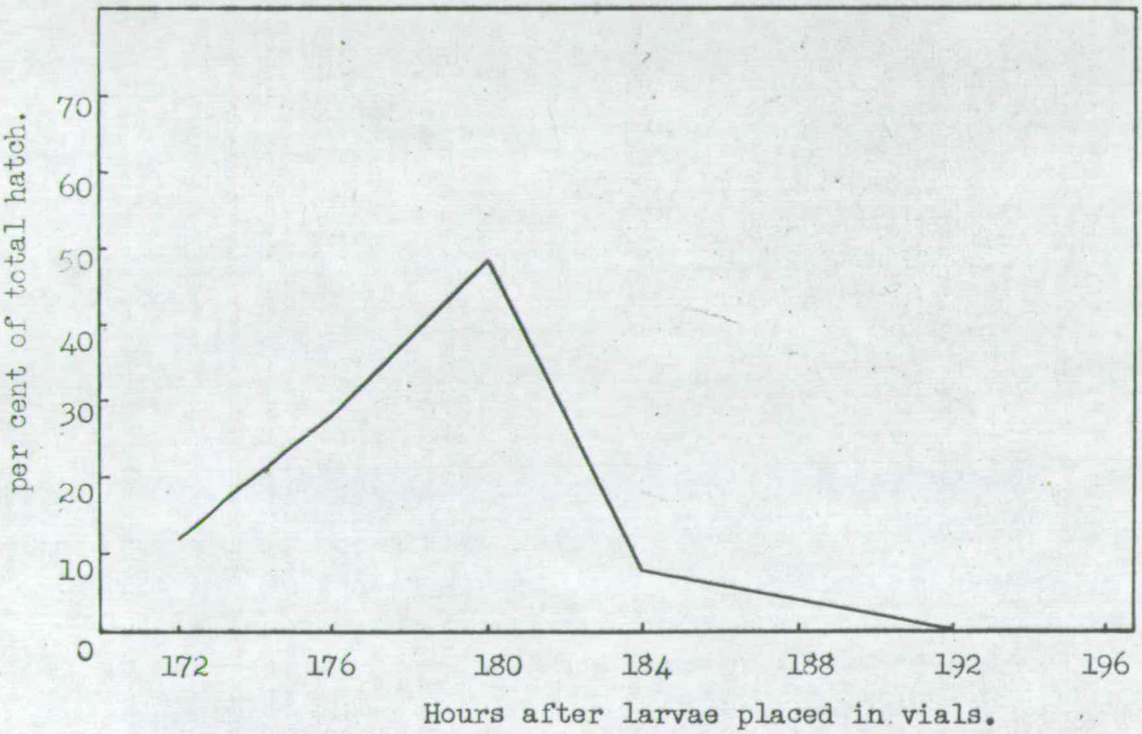


Figure 13. Age distribution of emergence of β_R/β_D flies.

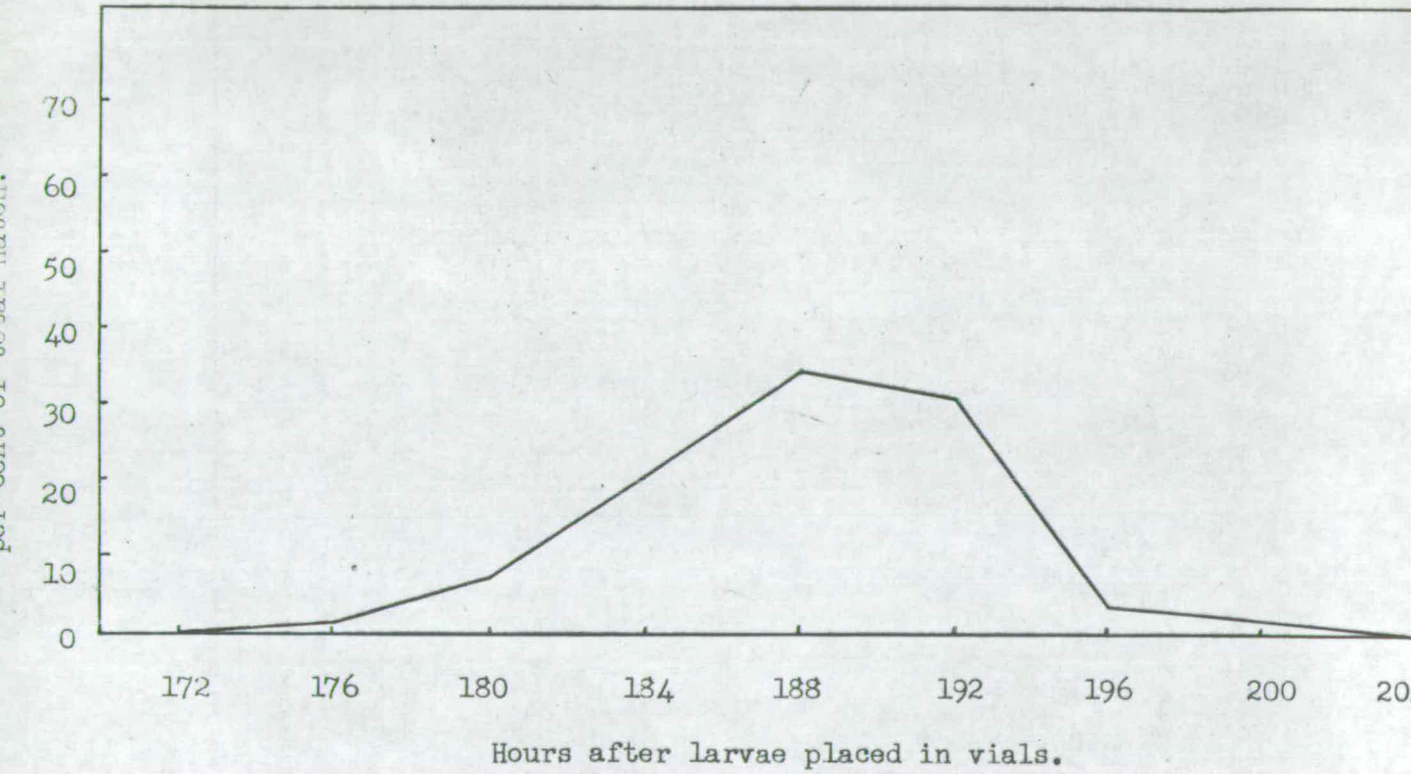


Figure 14. Age distribution of emergence of β_R/β_R flies.

Mean length of time from first larval instar to emergence (in hours)			
β_D/β_D	β_D/β_R	β_R/β_D	β_R/β_R
182.24 ± 0.44	182.64 ± 0.42	182.56 ± 0.37	188.08 ± 0.43

TABLE 15. Mean length of time from first larval instar stage until emergence.

genotypes was measured on the fourth to eighth days after emergence. This was used as a measure of fecundity.

Hiraizumi and Crow (1960) have reported that the amount of larval crowding has a significant effect on female fertility, so an attempt was made to raise the females under as uniform conditions as possible.

The females were collected on emergence and mated to males of the same genotype. The males were removed after two days and the females fed on yeast to stimulate them to lay. This was necessary as the number of eggs laid by females not treated in this way were very few, although this number probably approximates more closely to that laid by females in the population experiments. Females were then placed singly in vials without food, which were inverted over watch glasses containing 10 ml. cornmeal-molasses-agar medium. The watch glasses were changed each day and the numbers of eggs laid were counted. The results are shown in Table 16, the eggs laid by 100 females of each genotype being recorded on each day. Heterozygous females laid significantly more eggs than homozygous females. Females of β_R/β_R genotype tended to lay less eggs than those homozygous for $\underline{\beta_D}$, but this difference was not significant.

A more important component of fitness than total number of eggs laid is the number of fertile eggs produced. To determine this it was necessary to compensate for differences in egg hatchability. The numbers of fertile eggs laid per day by females of the different genotypes over the five day period examined are shown in Table 17. It can be seen that heterozygous females lay about twice as many fertile eggs as homozygous females.

Day after emergence	Genotype of female			
	β_D/β_D	β_D/β_R	β_R/β_D	β_R/β_R
4	19.13 ± 1.44	16.43 ± 1.32	22.71 ± 1.24	18.92 ± 1.81
5	26.87 ± 1.76	29.59 ± 1.45	32.47 ± 1.49	21.29 ± 1.52
6	28.24 ± 1.35	44.12 ± 1.37	47.91 ± 1.73	24.86 ± 1.57
7	21.89 ± 1.43	39.78 ± 1.82	35.32 ± 1.17	17.79 ± 1.38
8	22.71 ± 1.21	23.34 ± 1.18	27.14 ± 1.44	17.11 ± 1.71

TABLE 16. Female fecundity.

Trait	Genotype of female			
	β_D/β_D	β_D/β_R	β_R/β_D	β_R/β_R
Mean number of eggs laid per day for 5 days	23.77	30.65	33.11	19.99
Mean number of fertile eggs laid per day	11.89	21.46	23.51	10.00

TABLE 17. Mean number of fertile eggs laid per day.

Earliness of reproduction is expected to be an important component of fitness, but no differences were observed, between genotypes, for this trait, the maximum number of eggs being laid on the sixth day after emergence in all cases.

(f) Longevity

Maynard Smith (1958) has shown that whether or not a female Drosophila is laying eggs has a distinct effect on her longevity, this being inversely related to the amount of egg laying. Virgin females will tend to live considerably longer than mated females. In populations the majority of females will have mated and be laying eggs, so only the longevity of mated females will be considered in this section.

The longevity of males and mated females of the four genotypes was determined. For each genotype, 200 flies of each sex were collected within twelve hours of emergence. The males were placed in groups of five in vials, and the females placed in vials, also five to a vial, with five males of the same genotype. The flies were shaken into vials containing fresh food every second day without etherisation. The number of flies living was determined every second day.

Survival curves for males are shown in Figure 15 and those for females in Figure 16. The mean lengths of survival for males and females of the four genotypes are given in Table 18. In all cases the mean length of survival of males was significantly longer than that of females. In males the heterozygotes were longer lived than either homozygote. This

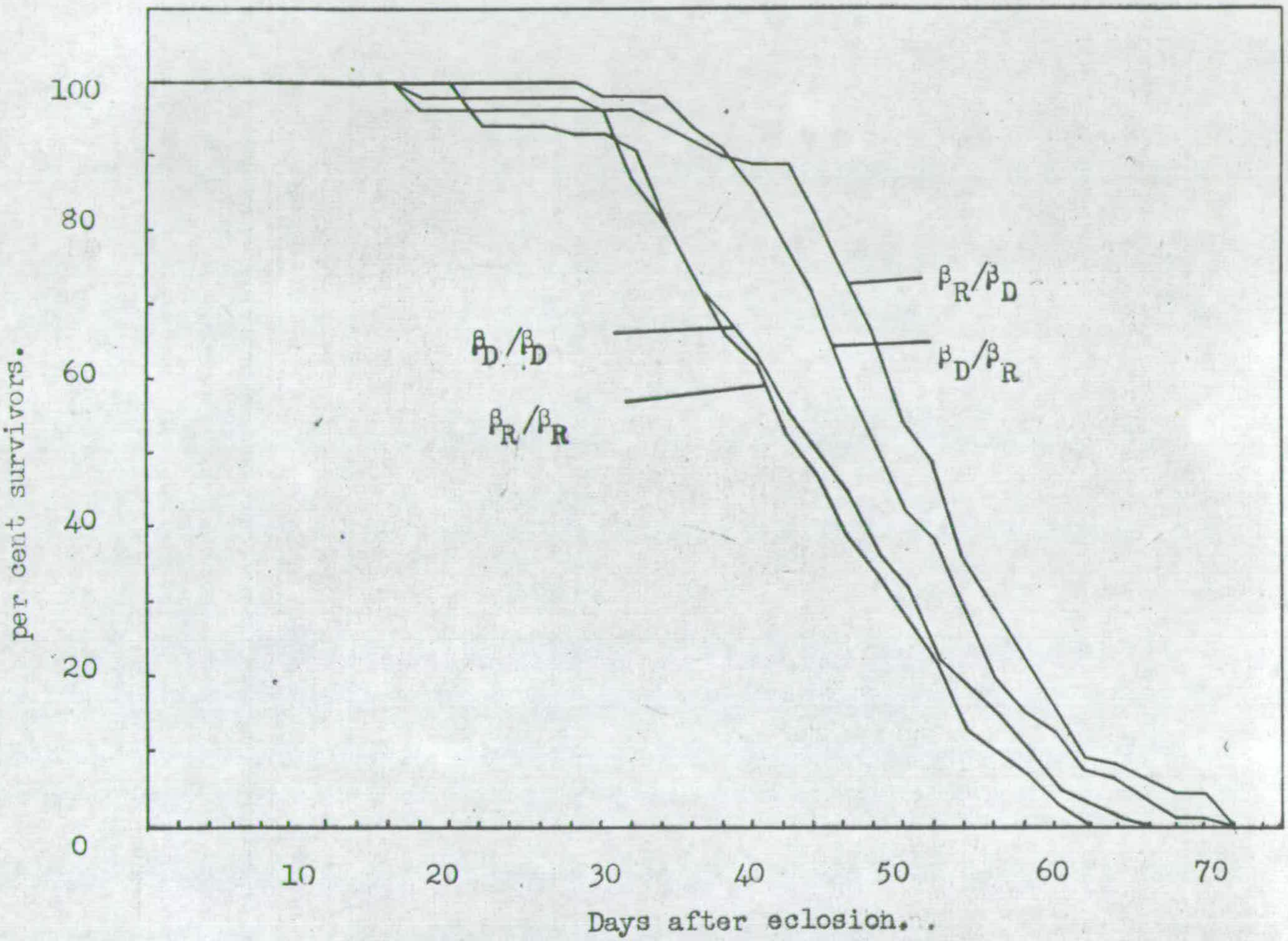


Figure 15. Survival curves for males.

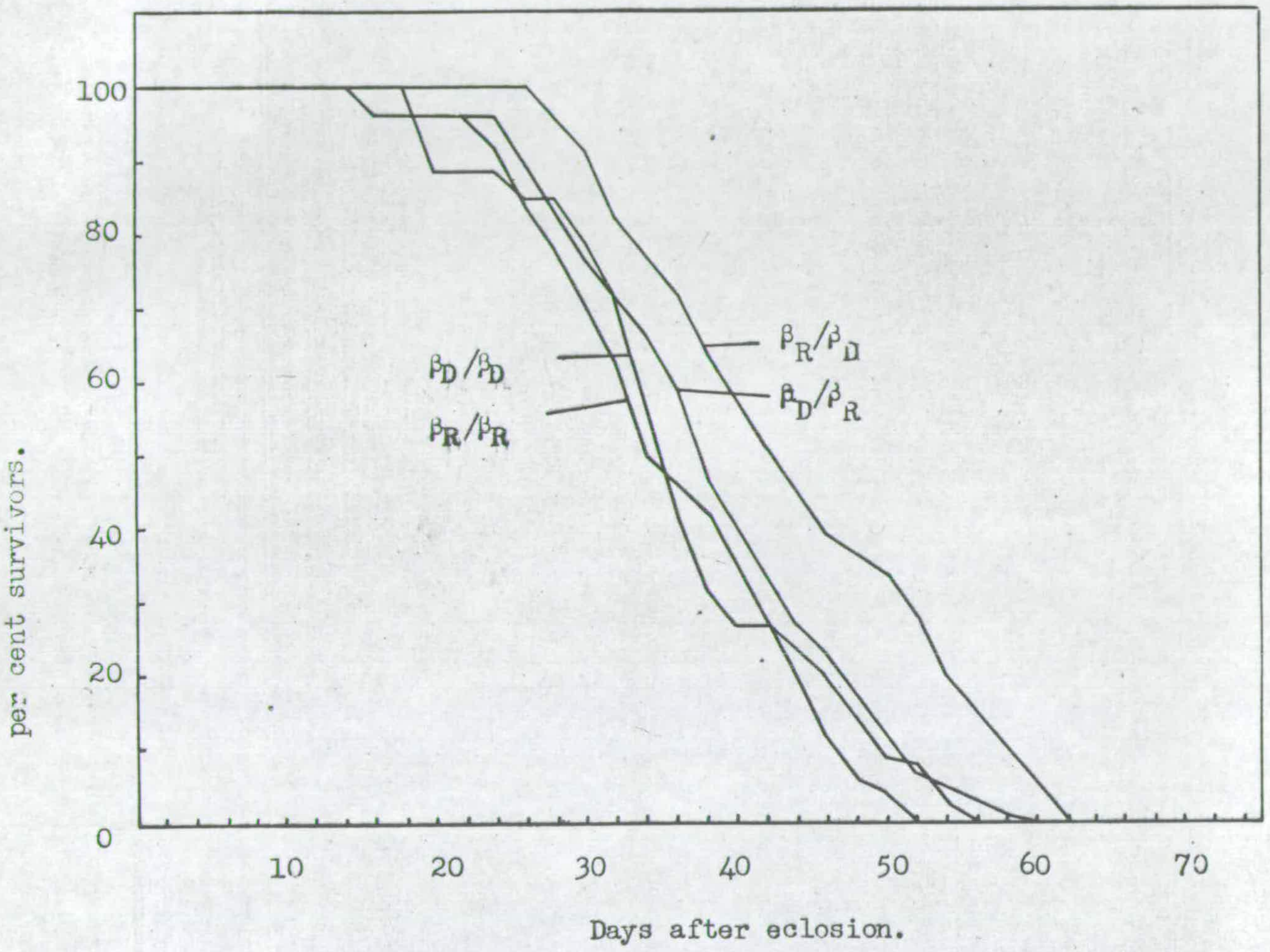


Figure 16. Survival curves for fertilised females.

Sex	Genotype			
Se	β_D/β_D	β_D/β_R	β_R/β_D	β_R/β_R
Male	43.09 ± 1.02	48.46 ± 0.93	50.18 ± 0.81	41.72 ± 0.99
Mated female	34.94 ± 0.98	37.46 ± 0.99	41.73 ± 1.01	33.60 ± 0.84

TABLE 18. Mean length of survival, in days, of males and mated females.

difference was highly significant. Such a difference was not quite so apparent in females. No significant differences were observed between homozygotes or between heterozygotes. The differences in longevity between β_R/β_D females and the two homozygotes were highly significant. Females of β_D/β_R genotype were longer lived than those of β_D/β_D or β_R/β_R genotypes, but this difference was just significant at the five per cent level in the former case. Thus some degree of heterozygote superiority was also apparent in this trait.

To summarise the studies on fitness, heterozygote superiority was found in the following traits:- viability from egg stage to eclosion, female fecundity and longevity. Thus this may be the mechanism responsible for maintaining the polymorphism.

It was possible to estimate the mean change in gene frequency per generation, Δq, from the rate of approach to equilibrium in bottle populations. Δq was found to equal 0.007. Assuming that heterozygote superiority was responsible for the maintenance of this polymorphism and that the relative selective values of the genotypes were the same at all gene frequencies, it was possible to determine the relative selective values of homozygotes and heterozygotes by the method of Wright and Dobzhansky (1946). If the selective value of heterozygotes was taken to be unity, the relative selective values of β_D/β_D and β_R/β_R homozygotes were equal and had a value of 0.68.

As longevity was not a component of total fitness in the bottle populations, the differences in fitness between homozygotes and heterozygotes are summarised in the number of fertile eggs laid per day by homozygous and heterozygous females (c.f. Table 17). Using this data, it was possible to calculate the relative selective values of homozygotes and heterozygotes. The selective value of each homozygote, compared to that of the heterozygotes taken as unity, was 0.50.

This estimate obtained from the fitness studies was less than that obtained from the data from the bottle populations. Thus some factors other than those considered in the fitness studies must be involved. It will be shown in the next section that genotype frequency has an effect on viability. The effect does not account for the difference in the estimates of the relative selective values, but it is possible that genotype frequency may have differential effects on other components of fitness of heterozygotes and homozygotes which would account for this difference. That this may be so is indicated by the finding that the changes in gene frequency over the first five generations of the bottle population, in which the initial frequency of $\underline{\rho_D}$ was high, are consistent with the estimates of relative selective values obtained from the fitness studies, while those in the population begun at a low frequency of $\underline{\rho_D}$ are not. In the latter case the differences in the relative selective values of homozygotes and heterozygotes would have to be smaller to account for the observed changes in gene frequency.

The importance of the heterozygote superiority in longevity observed in these studies was not clear. In cage populations where longevity was a component of total fitness, it was to be expected that the superiority of heterozygotes in this trait would accelerate the rate of change in gene frequency towards the equilibrium point. However, the rate of approach to an equilibrium point was similar in bottle and cage populations. Thus the difference in longevity of hetero-

:zygotes and homozygotes cannot be large enough to have an effect on the rate of change in gene frequency. In these studies, longevity was determined under ideal conditions, where competition for food was at a minimum. Flies kept under these conditions will be longer lived than those in the experimental populations. Therefore the differences in longevity may have been magnified under these ideal conditions and are not so great in the cage populations.

4. <u>The effect of genotype frequency on viability</u>

In the previous section some degree of overdominance was demonstrated in a number of traits connected with total fitness, these being viability from egg stage to adult, female fecundity and longevity. These traits were studied separately and the assumption was made that these were a guide to the relative adaptedness of the genotypes in the population. It is possible, however, that the interaction of genotypes in the population may have some effect on their relative adaptive values. The frequency of the various genotypes in the population may have an effect on the relative fitness of these genotypes.

The work of Lewontin (1955) and Kojima and Yarborough (1966) showed that genotype frequency had an effect on viability. The latter workers postulated some type of frequency dependent selection was acting, such that selection was at a minimum at the equilibrium frequency found in the population, and this mechanism was responsible for the maintenance of genes at an intermediate frequency. It is difficult to postulate a convincing mechanism for this type of selection in *Drosophila*. One possibility is that there is a type of larval interaction, such that each genotype relies on the others present for the supply of some necessary substance and the efficiency of the system is maximised at the equilibrium frequency.

In view of the differences between the hatchability of eggs of the various genotypes, the viability from first larval instar stage to eclosion was determined. Larvae of the three genotypes, β_D/β_D, β_D/β_R and β_R/β_R, were collected as described previously and placed in bottles. A density of 400 larvae per bottle was used. The proportions of the three types of larvae placed in the bottles simulated Hardy-Weinberg ratios for the seven $\underline{\beta_D}$ frequencies examined. These $\underline{\beta_D}$ frequencies and the expected Hardy-Weinberg genotype frequencies are shown in Table 19. The larvae developed at 25 ± 1°C and females were scored every day after the first emergence. It was necessary to cross all LF females to DF to determine if these were homozygous or heterozygous for $\underline{\beta_D}$. The observed genotype frequencies are shown in Table 20. The ratio of observed to expected genotype frequency was used as a measure of viability, and the viabilities are shown in Table 21.

The observed genotype frequencies were significantly different from expected at $\underline{\beta_D}$ frequencies 0.10, 0.25, 0.50 and 0.75. With the exception of 0.50, these frequencies are very different from

Frequency of β_D	Expected proportion		
	β_D/β_D	β_D/β_R	β_R/β_R
0.10	0.01	0.18	0.81
0.25	0.06	0.38	0.56
0.40	0.16	0.48	0.36
0.50	0.25	0.50	0.25
0.60	0.36	0.48	0.16
0.75	0.56	0.38	0.06
0.90	0.81	0.18	0.01

TABLE 19. Expected proportions of β_D/β_D, β_D/β_R and β_R/β_R genotypes, under the assumption of no viability differences.

Expected frequency of β_D	Observed frequency of β_D	Observed proportion		
		β_D/β_D	β_D/β_R	β_R/β_R
0.10	0.135	0.03	0.21	0.76
0.25	0.30	0.13	0.34	0.52
0.40	0.425	0.17	0.51	0.33
0.50	0.385	0.20	0.37	0.42
0.60	0.575	0.33	0.49	0.18
0.75	0.695	0.42	0.55	0.04
0.90	0.92	0.84	0.16	0.00

TABLE 20. Observed proportions of β_D/β_D, β_D/β_R, β_R/β_R genotypes.

Frequency of β_D	Genotype		
	β_D/β_D	β_D/β_R	β_R/β_R
0.10	3.00	1.17	0.94
0.25	2.17	0.89	0.93
0.40	1.06	1.06	0.92
0.50	0.80	0.74	1.68
0.60	0.92	1.02	1.13
0.75	0.75	1.45	0.67
0.90	1.03	0.89	0.66

TABLE 21. Viability estimates.

those found in the experimental populations. A trend was apparent in the viabilities of genotype β_D/β_D. The equilibrium frequency of β_D found in the experimental populations was about 0.50. Below this frequency, the viabilities of this genotype were greater than one, while, above it, they were less than one, indicating selection against this genotype at β_D frequencies above the equilibrium one and selection for it at those frequencies below this.

No such trend was apparent in the viabilities of genotype β_R/β_R.

The viabilities of the three genotypes at an expected β_D frequency of 0.50 are interesting. The viability of genotype β_R/β_R was greater than one, while those of the heterozygote and the other homozygote were less. In the previous section it was shown that the β_R/β_R genotype tended to be less fit than β_D/β_D for a number of the traits studied, although not always significantly so. This increase in viability, at what was found to be the equilibrium gene frequency in the experimental populations, may compensate for this so that the selective values of the two homozygotes are approximately equal, as they must be if the equilibrium gene frequency is 0.50.

It was concluded that genotype frequency did have some effect on viability, but no general trend was apparent.

Studies on the fourth chromosome locus with an effect on abdominal pigmentation

The effect of the two alleles, γ_D and γ_R, at this locus on pigmentation has been described previously. Flies homozygous for γ_R have reduced number of bristles at the sternopleural, ocellar and abdominal sites (c.f. Table 1). The fitness of these flies is also markedly reduced. In a cage population in which fourth chromosomes containing γ_D and γ_R were segregating in a *DF* background, the frequency of the recessive allele declined rapidly from an initial frequency of 0.60. When this population was last examined, by crossing individual females to *DF* males, the frequency of γ_R was about 0.06.

The reduced fitness of γ_R/γ_R males and females was also apparent from the following crosses:

(i) γ_D/γ_R and γ_R/γ_R males x γ_R/γ_R females

(ii) γ_R/γ_R males x γ_D/γ_R and γ_R/γ_R females

The genetic background of all flies was *DF*. All flies used in these crosses were approximately the same age. In both types of cross, preadult viability of γ_D/γ_R and γ_R/γ_R will be reflected in the number of progeny of each type produced. In cross (i) there is competition between males and in cross (ii) the difference between females of the two genotypes is measured.

The results from crosses (i) and (ii) are shown in Table 22, only females being scored. A highly significant excess of *BF* females was observed in both types of cross, indicating that γ_R, when homozygous, reduces male fitness, as measured by competitive mating ability, and female fecundity. The fact that the number of *BF* females in the

Cross	Number of BF females		Number of SF females	
	Observed	Expected	Observed	Expected
γ_D/γ_R and γ_R/γ_R males x γ_R/γ_R females	549	241.5	417	724.5
γ_R/γ_R males x γ_D/γ_R and γ_R/γ_R females	613	291.75	554	875.25

TABLE 22. The numbers of BF and SF females emerging from the above crosses.

progeny of these crosses was more than 50 per cent would suggest that there is a reduction in the preadult viability of flies homozygous for γ_R. That this is the case can be seen by looking at the segregation ratios in Table 23, obtained when flies heterozygous for γ_R were crossed to those homozygous for this gene. The numbers of *BF* and *SF* females emerging from these crosses should be approximately equal if there were no differences in preadult viability. The fact that a significant excess of *BF* females was observed indicated that there was a reduction in the preadult viability of flies homozygous for γ_R.

The results from crosses (i) and (ii) are open to another interpretation. The excess of *BF* females found in these crosses could be due to the superior fitness of the heterozygotes, and not to a reduction in fitness of flies homozygous for γ_R.

To clarify the situation, four backcross lines were set up. In two of these lines, which will be referred to as 'γ_D backcrosses', flies homozygous and heterozygous for γ_D were backcrossed to γ_D/γ_D flies in each generation, either through males or through females. As the gene γ_D is dominant with respect to banding, it was necessary to use a fourth chromosome marker to distinguish flies homozygous for γ_D from those heterozygous for this gene. A fourth chromosome containing the markers cubitus interruptus (ci) and eyeless Russian (ey^R), in association with the gene γ_D, was used. As crossing over in the fourth chromosome is a rare event, these markers will remain associated with γ_D for a number of generations. This marked chromosome will be referred to as *ci ey*^{R} and flies homozygous for this chromosome as *ci ey*^{R} homozygotes. As the differences in

	Number of *BF* females		Number of *SF* females	
CROSS	Observed	Expected	Observed	Expected
γ_D/γ_R males x γ_R/γ_R females	189	150.5	112	150.5
γ_R/γ_R males x γ_D/γ_R females	204	170.5	137	170.5

TABLE 23: The numbers of *BF* and *SF* females emerging from the above crosses.

pigmentation pattern are only observable in females, the use of a marked fourth chromosome made the detection of males homozygous for γ_D possible. Thus, in one 'γ_D backcross' line, males homozygous and heterozygous for the *ci ey*^{R} chromosome were backcrossed to *ci ey*^{R} homozygotes, and, in the other line, females homozygous and heterozygous for the *ci ey*^{R} chromosome were backcrossed to males homozygous for this chromosome.

In the other two backcross lines, which will be referred to as 'γ_R backcrosses', flies heterozygous for the *ci ey*^{R} chromosome and those homozygous for γ_R were backcrossed to flies homozygous for γ_R in each generation, backcrossing being through males in one line and through females in the other.

A line in which the *ci ey*^{R} chromosome was segregating in a *DF* background was used to initiate the four backcross lines. Thus, flies heterozygous for this chromosome will be heterozygous for the two alleles at the γ locus. To minimise fluctuations in gene frequency due to drift, each line was made up of four bottle populations, the progeny of which were equally divided into another four bottles to form the next generation.

In the 'γ_R backcross' lines, no marker was present on the fourth chromosome containing γ_R and, as the effects of the genes at the γ locus on abdominal pigmentation were limited to females, it was necessary to find some way of distinguishing males heterozygous for γ_R from those homozygous for this gene. This was done by bristle counts. A bottle population was set up, the males emerging from which would be of two types, homozygous and heterozygous for γ_R. Counts of bristles at the sternopleural and ocellar sites were made on each male. The males were then tested for genotype by crossing to DF and observing the female progeny. From Table 24 it can be seen that males in which the sternopleural bristle score was less than 10 and the ocellar bristle score less than 6, were always homozygous for γ_R. By utilising both bristle scores it was possible to classify the genotypes of more males than on the basis of either score alone.

The appearance of the band on the sixth abdominal tergite of females heterozygous for the ci ey^R chromosome was less variable than in those females heterozygous for a fourth chromosome containing γ_D alone, when the genetic background was DF. This made the distinction between BF and SF females in the 'γ_R backcross' lines less susceptible to error.

To summarise, four backcross lines were set up, the genetic background in all lines being DF. In all lines, the flies used to set up any one generation of backcrossing were all taken from the previous generation.

(a) 'γ_D backcross' lines

(i) ci ey^R/ ci ey^R males were crossed to ci ey^R/ci ey^R and ci ey^R/γ_R females in each generation.

(ii) ci ey^R/ci ey^R females were crossed to ci ey^R/ci ey^R and ci ey^R/γ_R males in each generation.

In these backcrosses, the expected frequencies of flies homozygous and heterozygous for γ_D can be calculated for each

Bristle score of males		Number of males tested	Number of ci ey^R/γ_R males	Number of γ_R/γ_R males
Sternopleural	Ocellar			
6	3	3		3
6	4	2		2
6	5	1		1
7	4	4		4
7	5	4		4
8	4	1		1
8	5	11		11
9	5	20		20
9	6	5	3	2
10	5	7	6	1
10 or over	6 or over	42	42	

TABLE 24. The results of testing males with the above bristle scores for genotype. Males in which the sternoplerural bristle score was less than 10 and the ocellar bristle score less than 6 were always homozygous for $\gamma_{\underline{R}}$.

generation of backcrossing, assuming random mating and equal viability and fertility of the different genotypes. These expected frequencies can be compared with those observed to determine if the above assumptions were valid. However, flies homozygous for γ_D in these backcross lines were also homozygous for the two markers *ci* and *ey*^{R} and *ci ey*^{R} homozygotes have a much reduced fitness compared to flies homozygous for γ_D only. In the line used to initiate the backcrosses, the *ci ey*^{R} chromosome was segregating in a *DF* background and an equilibrium point was reached with the frequency of γ_R at 0.60. In a line in which the *ci ey*^{R} chromosome was segregating in a *DF* background and all *ci ey*^{R} homozygotes were removed in each generation, the same frequency of γ_R was reached, indicating that *ci ey*^{R} homozygotes have a very low selective value. An attempt was made to estimate this selective value by comparing the observed proportion of *ci ey*^{R} homozygotes in the *F2* of a cross between *ci ey*^{R} homozygotes and flies homozygous for γ_D, with that expected. The selective value obtained from this data was 0.29. It must be noted that this selective value only takes into account differences in viability of the two genotypes.

By correcting the expected frequencies of flies homozygous for γ_D for the reduced viability of *ci ey*^{R} homozygotes, it was possible to compare these expected frequencies with the observed frequencies of *ci ey*^{R} homozygotes in each generation of backcrossing. The expected frequency of *ci ey*^{R} homozygotes in each generation was calculated as follows:-

Consider the 'γ_D backcross' line (i) in which males homozygous for the *ci ey*^{R} chromosome were crossed to females homozygous and heterozygous for this chromosome in each generation. Let the frequency of females homozygous for the *ci ey*^{R} chromosome in any generation be P and the frequency of females heterozygous for this chromosome be Q. If there was random mating and no differences in viability and fertility among the different genotypes, the frequency of *ci ey*^{R} homozygotes expected in the next generation of backcrossing would be $(P + {}^1/_2\,Q)$. However, it has been shown that the viability of *ci ey*^{R} homozygotes is only 0.29 of that of flies homozygous for γ_D alone. The frequency of *ci ey*^{R} homozygotes in the next generation, taking into account this lowered viability, will be $0.29\,(P + {}^1/_2\,Q)$. The frequency of flies heterozygous for the *ci ey*^{R} chromosome will be ${}^1/_2\,Q$. These frequencies were corrected to give a total of unity. Thus the frequency of *ci ey*^{R} homozygotes expected in the next generation of backcrossing will be

$$\frac{[0.29\,(P + {}^1/_2\,Q)]}{[0.29\,(P + {}^1/_2\,Q)] + {}^1/_2\,Q}$$

and the frequency of flies heterozygous for the *ci ey*^{R} chromosome

$$\frac{{}^1/_2\,Q}{[0.29\,(P + {}^1/_2\,Q)] + {}^1/_2\,Q}$$

The observed and expected frequencies of females homozygous for the *ci ey*^{R} chromosome in the two 'γ_D backcrosses' are shown in Figures 17 and 18, for 30 generations of backcrossing. In both backcrosses the observed frequencies were slightly higher than expected, but not significantly so. As no significant differences were found between the observed frequencies and the expected frequencies corrected for viability differences using the above selective value, this would indicate that, in *ci ey*^{R} homozygotes, viability is much reduced but male fitness and female fertility are not reduced, compared to flies homozygous for γ_D only. This being so, if flies heterozygous for γ_D were fitter than those homozygous for this allele, the observed frequency of flies homozygous for the *ci ey*^{R} chromosome, and therefore for γ_D, would be lower than the expected frequency calculated assuming random mating and selection on viability but not on adult fitness. This was not found and it can therefore be inferred that flies homozygous for γ_D were not less fit than those heterozygous for this allele.

(b) 'γ_R backcross' lines

(i) γ_R/γ_R males were crossed to γ_R/γ_R and ci ey$^{R}/\gamma_R$ females in each generation. Males homozygous for γ_R were distinguished from those heterozygous for this allele by sternopleural and ocellar bristle scores.

(ii) γ_R/γ_R females were crossed to γ_R/γ_R and ci ey$^{R}/\gamma_R$ males in each generation.

Figure 17. The expected and observed frequencies of ci ey^R/ci ey^R females in the '$\underline{\gamma_D}$ backcross' line in which males homozygous and heterozygous for the $\underline{\text{ci ey}^R}$ chromosome were backcrossed to females homozygous for this chromosome. Upper line represents the observed frequencies. Lower line represents the expected frequencies.

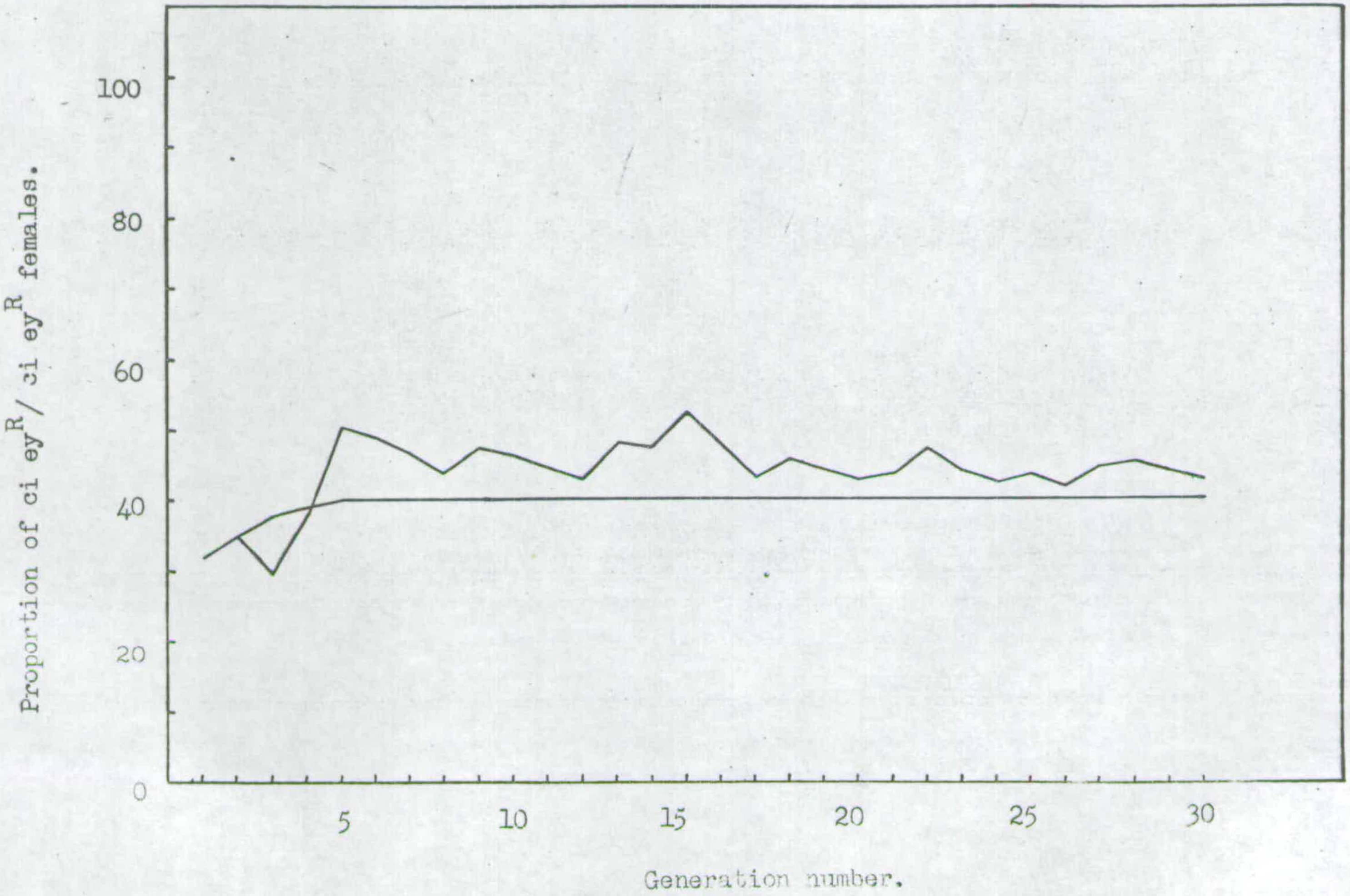
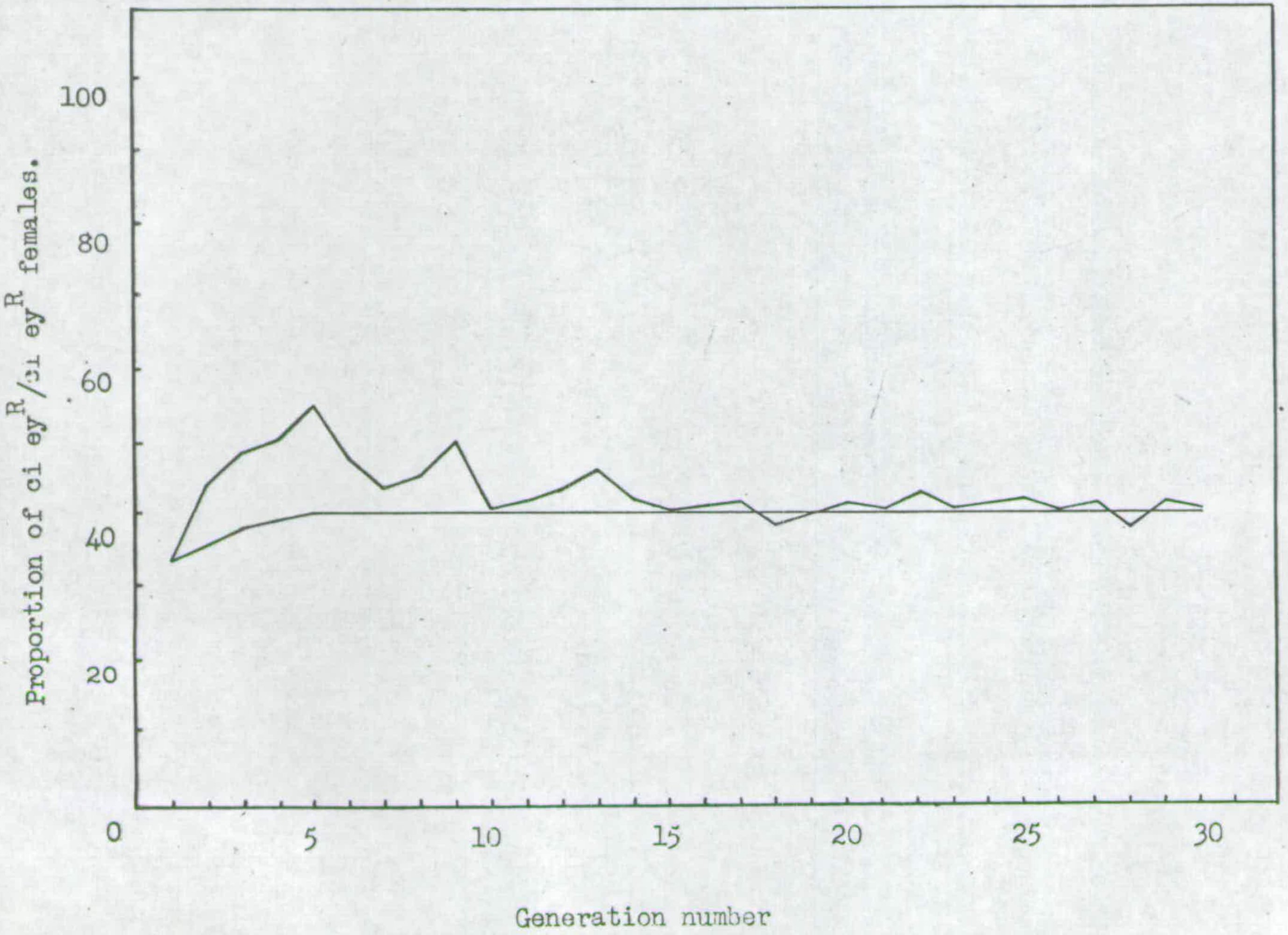


Figure 18. The expected and observed frequencies of ci ey^R/ ci ey^R females in the 'γ_D backcross' line in which females homozygous and heterozygous for the ci ey^R chromosome were backcrossed to males homozygous for this chromosome.

Upper line represents the observed frequencies.

Lower line represents the expected frequencies.



The expected frequencies of flies homozygous and heterozygous for $\underline{\gamma_R}$ were calculated for each generation of backcrossing, assuming random mating and equal viability and fertility of the two genotypes. The frequencies of γ_R/γ_R females expected on this basis and the frequencies of γ_R/γ_R females observed in the two '$\underline{\gamma_R}$ backcross' lines are shown in Figures 19 and 20, for 30 generations of backcrossing. The observed frequencies were significantly lower than those expected. After 30 generations of backcrossing the observed frequency of γ_R/γ_R females was 0.78 in line (i) and 0.67 in line (ii). In both lines the differences between observed and expected frequencies will reflect differences in preadult viability. In line (i) the difference between females homozygous and heterozygous for $\underline{\gamma_R}$ is being measured, while in line (ii) there is competition between males. Thus males and females homozygous for $\underline{\gamma_R}$ were less fit than those heterozygous for this gene, this reduction in fitness being greater in males than in females, as the observed frequency of γ_R/γ_R females was lower in line (ii) than in line (i).

It was concluded that flies homozygous for the recessive allele at the fourth chromosome locus were less fit than those heterozygous or homozygous for $\underline{\gamma_D}$. This reduced fitness was due to a reduction in male and female fitness. To determine if this reduction in fitness only affected some components of total fitness a number of traits were studied. These were the proportion of sterile matings, competitive mating ability of males, female fecundity and viability

Figure 19. The expected and observed frequencies of γ_R/γ_R females in the '$\gamma_{\underline{R}}$ backcross' line in which male homozygous and heterozygous for $\gamma_{\underline{R}}$ were backcrossed to females homozygous for this allele.

Upper line represents the expected frequencies.

Lower line represents the observed frequencies.

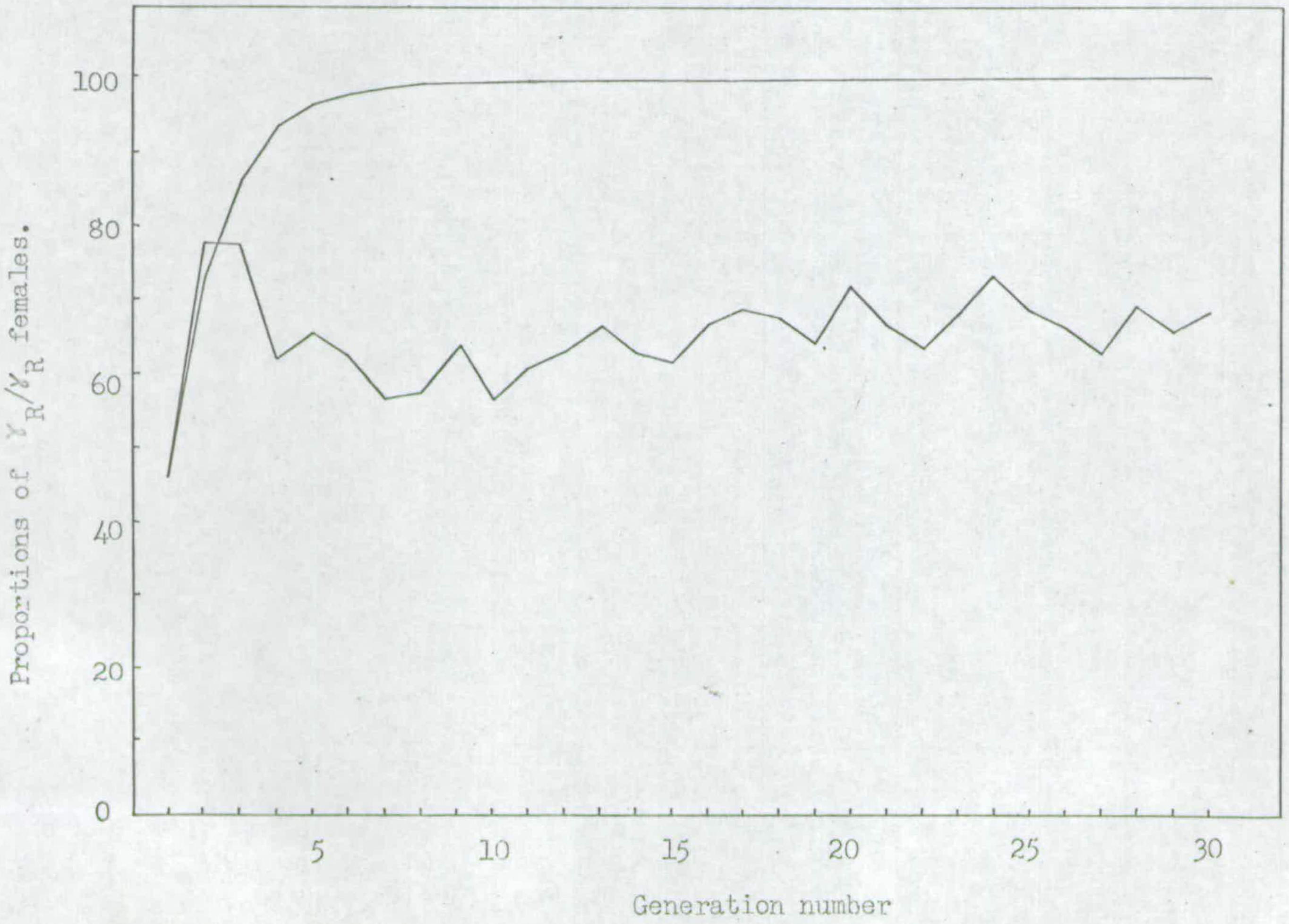
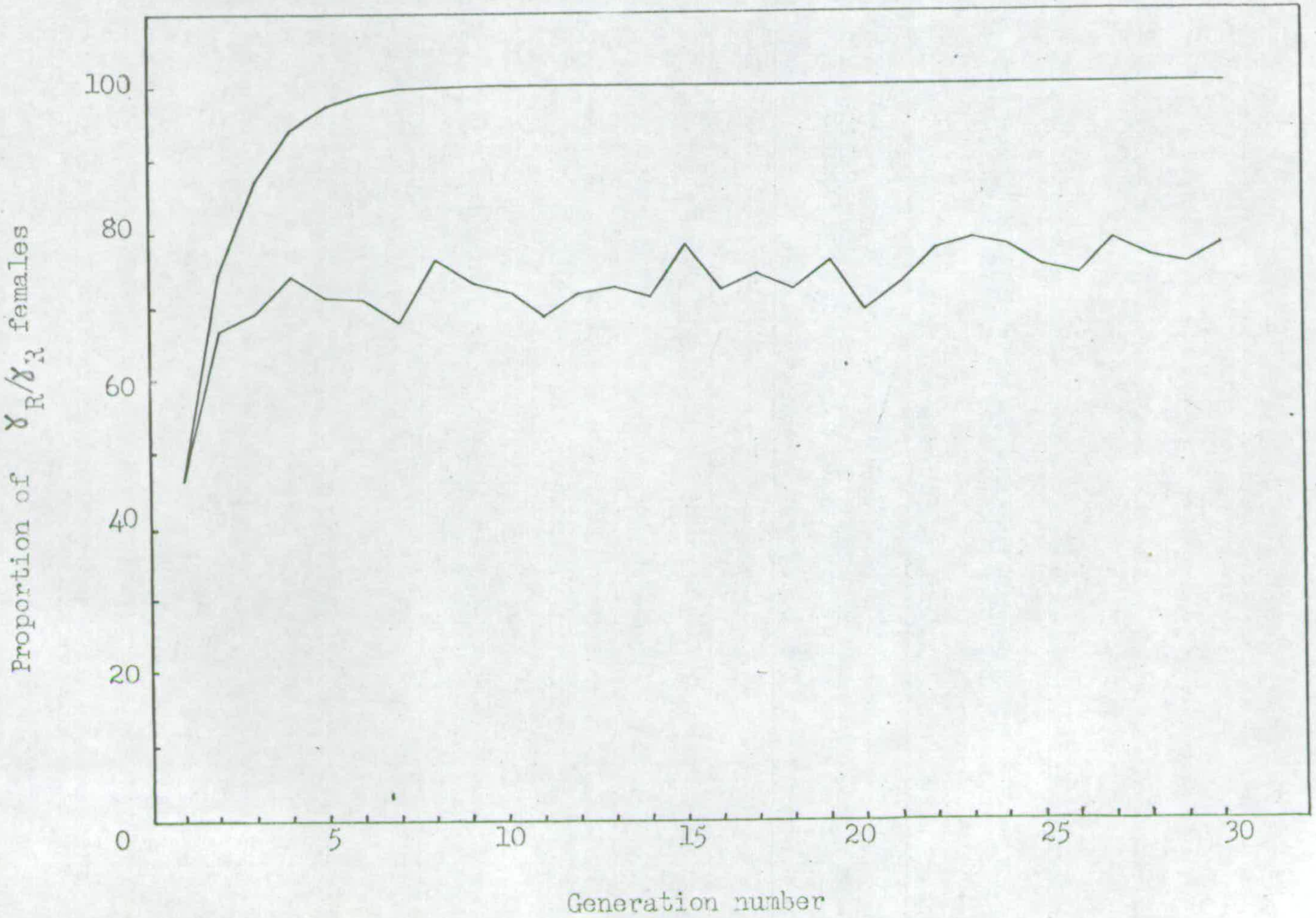


Figure 20. The expected and observed frequencies of γ_R/γ_R females in the '$\underline{\gamma_R}$ backcross' line in which females homozygous and heterozygous for $\underline{\gamma_R}$ were backcrossed to males homozygous for this allele. Upper line represents the expected frequencies. Lower line represents the observed frequencies.



during two stages of development. The methods used in the study of these traits have been outlined in the previous section.

Comparisons of the four karyotypes, γ_D/γ_D, γ_D/γ_R, γ_R/γ_D and γ_R/γ_R were made, γ_D/γ_R representing the heterozygote from a mating in which the genotype of the female parent was γ_R/γ_R, and γ_R/γ_D representing that in which it was γ_D/γ_D. The two types of heterozygote were included in these studies to determine if any maternal effect was present. The genetic background, in all cases, was *DF*.

The proportion of sterile matings was determined for the pair matings shown in Table 25. The results are shown in this table, each result being based on 200 pair matings. The sterility in crosses in which neither parent was homozygous for γ_R, was very low. The largest proportion of sterile matings was found when γ_R/γ_R males were mated to females of the same genotype. This proportion was comparable to the values in Table 10, the results from pair matings of flies homozygous and heterozygous for the two alleles at the *p* locus on the third chromosome. The genetic background in these experiments was *DF*, so all flies were homozygous for γ_R. Thus this gene has a considerable effect on sterility when homozygous. Both males and females are affected, as some sterility was found in those crosses in which the male or female parent was homozygous for γ_R.

Cross (♂ x ♀)	Percentage of sterile matings
γ_D/γ_D x γ_D/γ_D	0.0
γ_D/γ_R x γ_D/γ_R	0.5
γ_R/γ_D x γ_R/γ_D	0.0
γ_R/γ_R x γ_R/γ_R	12.0
γ_R/γ_R x γ_D/γ_D	4.5
γ_R/γ_R x γ_D/γ_R	4.5
γ_R/γ_R x γ_R/γ_D	4.0
γ_D/γ_D x γ_R/γ_R	5.0
γ_D/γ_R x γ_R/γ_R	4.5
γ_R/γ_D x γ_R/γ_R	5.5

TABLE 25: Proportion of sterile matings.

Competitive mating ability of males was determined in the same way as for the β locus. The results are shown in Table 26. It was necessary to examine the female progeny of these crosses to determine the genotype of the male parent. γ_R/γ_R males were significantly less successful in gaining mates. When the genotypes of the competing males were γ_D/γ_D and γ_R/γ_R, it was possible to detect if a double mating had occurred as the female progeny would be of two types. When males heterozygous and homozygous for γ_R competed, in some crosses containing *BF* and *SF* female progeny, the number of *SF* females in the progeny was greater than expected if only the heterozygote had mated. These were presumed to be double matings and are recorded as such in Table 26. In all crosses in which one of the competing males was homozygous for γ_R, double matings occurred. It is possible that γ_R/γ_R males do not produce a large number of viable sperm, as it has been shown that females, after mating to a male producing few active sperm, are willing to remate within a very short time.

Female fecundity was determined by counting the number of eggs laid on the fourth to eighth days after emergence. 100 females of each genotype were examined and the results are shown in Table 27. Significantly fewer eggs were laid by females homozygous for γ_R. γ_D/γ_R females laid fewer eggs than γ_R/γ_D or γ_D/γ_D females, indicating some type of maternal effect.

Viability was determined for two intervals of development, from first larval instar to eclosion and from egg stage to eclosion. The

Genotype of male parent				
γ_D/γ_D	γ_D/γ_R	γ_R/γ_D	γ_R/γ_R	Double matings
104	96			
98		102		
172			8	20
	175		7	18*
		166	12	22*

TABLE 26: Competitive mating ability of males. 200 pairs of males tested in each case.

* presumed double matings.

Day after emergence	Genotype of female			
	γ_D/γ_D	γ_D/γ_R	γ_R/γ_D	γ_R/γ_R
4	29.78 ± 1.34	25.62 ± 1.56	27.43 ± 1.29	17.12 ± 1.76
5	42.91 ± 1.53	34.83 ± 1.49	43.34 ± 1.42	22.35 ± 1.63
6	59.62 ± 1.46	46.21 ± 1.72	57.82 ± 1.64	25.41 ± 1.54
7	51.32 ± 1.48	41.63 ± 1.42	50.21 ± 1.39	19.21 ± 1.67
8	34.74 ± 1.31	32.04 ± 1.47	36.28 ± 1.52	17.58 ± 1.82

<u>TABLE 27:</u> Female fecundity.

results are shown in Tables 28 and 29. The viability from first larval instar to eclosion was singificantly lower for genotypes γ_D/γ_R and γ_R/γ_R than for the other two genotypes. Thus the female parent appears to have some effect on the viability of her progeny, as well as on the egg laying ability of her female progeny. This effect could be a simple maternal one, or may be due to some type of cytoplasmic inheritance.

The viability from egg stage to eclosion was significantly lower for γ_R/γ_R.

Egg hatchabilities, estimated from the above results, are shown in Table 30. γ_R/γ_R eggs had a lower hatchability than the eggs of the other three genotypes.

No determination of rates of development were made, but the first females hatching from bottles containing flies homozygous and heterozygous for $\underline{\gamma_R}$, always showed the *BF* phenotype. Thus flies heterozygous for $\underline{\gamma_R}$ may develop faster than those homozygous for this gene.

It is of interest to look at the results in Table 22 in view of the effects of $\underline{\gamma_R}$ when homozygous on various components of fitness. Preadult viability and female fecundity are both reduced by approximately one half in flies homozygous for $\underline{\gamma_R}$ compared to γ_D/γ_R heterozygotes. On this basis a 1:1 ratio of *BF* to *SF* female progeny is expected when γ_D/γ_R and γ_R/γ_R

Genotype			
γ_D/γ_D	γ_D/γ_R	γ_R/γ_D	γ_R/γ_R
25.37 ± 0.43	21.77 ± 0.84	26.83 ± 0.39	20.33 ± 0.76
24.87 ± 0.38	20.97 ± 0.75	26.13 ± 0.41	18.87 ± 0.69
26.03 ± 0.45	20.37 ± 0.72	25.83 ± 0.46	20.67 ± 0.64

TABLE 28: Viability from first larval instar stage to eclosion. Mean number of adults hatching from 30 larvae.

Genotype			
γ_D/γ_D	γ_D/γ_R	γ_R/γ_D	γ_R/γ_R
25.13 ± 0.48	20.73 ± 1.01	23.73 ± 0.43	11.13 ± 1.25
23.87 ± 0.42	21.03 ± 0.65	24.43 ± 0.51	8.97 ± 0.95
24.23 ± 0.39	20.33 ± 0.71	24.97 ± 0.52	9.57 ± 0.87

Table 29: Viability from egg stage to eclosion. Mean number of adults hatching from 30 eggs.

Trait	Genotype			
	γ_D/γ_D	γ_D/γ_R	γ_R/γ_D	γ_R/γ_R
Mean number of adults hatching from 30 first instar larvae	25.42	21.04	26.26	19.96
Mean number of adults hatching from 30 eggs	24.41	20.70	24.38	9.89
Egg hatchability	0.96	0.98	0.93	0.49

Table 30: Egg hatchability

females competed. The results in Table 22 do not differ significantly from a 1:1 ratio. Taking into account the differences in male mating ability and preadult viability of flies homozygous for $\underline{\gamma_R}$ and γ_D/γ_R heterozygotes, the numbers of *BF* and *SF* female progeny observed in crosses in which γ_D/γ_R and γ_R/γ_R males were competing were not significantly different from those expected on this basis.

In all the components of fitness studied, the gene $\underline{\gamma_R}$ had a deleterious effect on fitness when homozygous. In view of this, it was to be expected that this allele would be at a low frequency in the *Kaduna* population, the base population from which the lines selected for sternopleural bristle number were derived. The Kaduna population had twice been sampled in an effort to determine the frequency of $\underline{\gamma_R}$. Dr. A. Robertson extracted ten fourth chromosomes and Dr. C. McPhee fourty eight, but all these contained the dominant allele $\underline{\gamma_D}$. A further one hundred and fifty fourth chromosomes were extracted by the method shown in Figure 21. All females examined showed the *BF* phenotype, the phenotype of females containing *Xa* in a *DF* background being *SF*. Thus all fourth chromosomes extracted contained the dominant allele at the $\underline{\gamma}$ locus. The recessive gene must be at a very low frequency in the *Kaduna* population, if indeed it is present at all. It is not impossible that $\underline{\gamma_R}$ was produced by mutation at some time during the selection procedure.

$$\begin{tikzcd}
\frac{K}{Y} ; \frac{K}{K} ; \frac{K}{K} ; \frac{K}{K} & \times \arrow[dl] & \frac{D}{D} ; \frac{Xa}{D ; D} ; \frac{Ci^{D}}{D} \quad \text{(mass mating)} \\
\frac{D}{Y} ; \frac{Xa}{K ; K} ; \frac{Ci^{D}}{K} & \times \arrow[d] & \frac{D}{D} ; \frac{D}{D} ; \frac{D}{D} ; \frac{D}{D} \quad \text{(pair matings)} \\
& \frac{D}{D} ; \frac{Xa}{D : D} ; \frac{D}{K} &
\end{tikzcd}$$

These females scored to determine if BF or SF

Figure 21. Extraction of fourth chromosomes from the Kaduna population.

K represents chromosomes from Kaduna.

D represents chromosomes from DF.

The effect of temperature on pigmentation pattern

The effect of temperature on the expression of genes affecting pigmentation of the sixth abdominal tergite was studied. The dense black pigment in the posterior abdominal tergites of the male obscures any effect these genes may have on pigmentation, so observations were confined to females. Flies were normally raised at 25°C and the expression of alleles at the ρ and γ loci in a DF background at this temperature has been described previously. The effects of a decrease in temperature on the expression of these alleles was studied by raising flies at 17°C and those due to an increase in temperature by studying flies raised at 30°C. The genetic background of all flies studied was DF.

The effects on pigmentation of raising flies, homozygous and heterozygous for the two alleles at the ρ locus at 17°C and 30°C are shown in Table 31. Temperature does not appear to affect the expression of ρ_D when homozygous, females with this genotype showing an absence of pigment in the sixth abdominal tergite at 17°C and 30°C. Females heterozygous for this gene have a faint band of brown pigment across the sixth tergite when raised at 17°C. The pigmentation pattern of females homozygous for the recessive allele, ρ_R, was affected at both high and low temperatures. These females were also homozygous for the recessive allele at the γ locus on the fourth chromosome, so any effect of temperature on pigmentation pattern of these females could involve one or both these genes. Females of this genotype raised at 17°C had a dark band of pigment across the sixth tergite in addition to

Temperature	Genotype				
	$\beta_D/\beta_D;\gamma_R/\gamma_R$	$\beta_D/\beta_R;\gamma_R/\gamma_R$	$\beta_R/\beta_R;\gamma_R/\gamma_R$	$\beta_R/\beta_R;\gamma_D/\gamma_D$	$\beta_R/\beta_R;\gamma_D/\gamma_R$
17°C	No dark pigment	Faint band of brown pigment	Broad band of pigment	Very broad band of pigment	Very broad band of pigment
25°C	No dark pigment	No dark pigment	No band of pigment	Broad band of pigment	Band of pigment of variable width
30°C	No dark pigment	No dark pigment	No band of pigment	Band of pigment	Faint band of pigment

TABLE 31: The effect of temperature on pigmentation of the sixth abdominal tergite in females with the genotypes shown. The genetic background of these females was DF.

the two spots present on this tergite at 25°C. This phenotype was indistinguishable from that of females homozygous for γ_D raised at 25°C, indicating that it may be the expression of the allele at the γ locus that was affected. In females raised at 30°C, no band was present and the spots on the sixth tergite were smaller than those in females of the same genotype raised at 25°C.

The effects of temperature on the expression of γ_D in females, homozygous and heterozygous for this gene, are also shown in Table 31. Both types of female had a broader band of pigment across the sixth tergite when raised at 17°C than females of the same genotype raised at 25°C. Heterozygotes were indistinguishable from homozygotes, there being no variability in the width of the band in the former. The variability in the width of the band was increased in females heterozygous for γ_D raised at 30°C. The band of pigment in γ_D/γ_D females raised at 30°C tended to be less broad than at 25°C.

In general, the amount of pigment in the sixth tergite was increased at lower temperatures and decreased at higher ones. The amount of melanin in the whole of the abdominal cuticle was increased in flies raised at 17°C and the broadening of the band in the sixth tergite of females homozygous and heterozygous for γ_D may be due to this general effect. In females homozygous for ρ_R and γ_R, the increase in the amount of pigment in the sixth tergite at 17°C was proportionately greater than in the other tergites, indicating some type of specific effect. As the resulting phenotype was similar to that in flies with dominant allele at the γ locus, it is probable that this effect is due to the recessive allele at this locus. The precise role of this locus in the production of melanin is not known, but temperature does affect the dominance relationships of the two alleles, γ_D and γ_R.

The dominant gene at the β locus, which removes pigment from the sixth tergite, was epistatic to the other genes producing pigment in this tergite. It is probable, therefore, that the effect of this locus was at an earlier step in the pathway leading to the production of melanin than those of the other loci. The effect of β_D does not appear to be reversible by temperature.

The largest observable effect of temperature on pigmentation of the sixth abdominal tergite was found in females homozygous for β_R and γ_R. This effect is probably due to the allele at the γ locus and was used in an attempt to determine the time during development at which this locus acts on pigmentation. Three series of vials containing fertilised females of the above genotype were set up. One series was kept as a control, all vials being kept at 25°C until the emergence of the progeny. In the second series, two vials were placed at 17°C for 24 hours on consecutive days of development, the rest of development taking place at 25°C. In the third series, two vials were transferred to 30°C for 24 hours on consecutive days.

The eggs, when laid in the vials, will be at different stages of development. Therefore, at any given time, the individuals in a particular vial will be at various stages of development. In order to observe the effect of temperature on individuals at approximately the same stage of development, only the first females hatching from the vials in each series were scored for pigmentation pattern of the sixth abdominal tergite. Thus only those individuals at the most advanced stage of development when the vials were removed to 17°C or 30°C were scored.

The results of these studies are shown in Table 32. Females placed at 17°C on the eighth day of development had a band of pigment across the sixth tergite. This band was not as broad as that in females of the same genotype raised at 17°C, but it does indicate that some effect was acting on pigmentation at this stage of development.

The amount of pigment in the abdominal cuticle can be increased after emergence. This was shown by raising females homozygous for $\underline{\beta_R}$ and $\underline{\gamma_R}$ at 25°C or 30°C and placing them at 17°C on hatching. Females treated in this way developed a band of pigment across the sixth tergite which was not apparent on emergence.

The reduction in the size of the spots in the sixth abdominal tergite, observed when females were raised at 30°C, was not a large enough effect to enable the time of development at which it was acting to be observed, females from all vials in the third series having the *SF* phenotype.

Day of Development	Stage of Development	Temperature	
		17°C	30°C
1	egg	-	-
2	larval	-	-
3	larval	-	-
4	larval	-	-
5	larval	-	-
6	pupal	-	-
7	pupal	-	-
8	pupal	Band of pigment across 6th tergite	-
9	pupal	-	-
10	imago	Band of pigment across 6th tergite	-

TABLE 32: The effect of 24 hours at 17°C or 30°C at various stages of development on the pigmentation pattern of the sixth abdominal tergite of females homozygous for $\underline{\beta_R}$ and $\underline{\gamma_R}$. - indicates that the phenotype of these females was the same as that at 25°C.

DISCUSSION

The studies on the two alleles at the $\underline{\beta}$ locus on the third chromosome have shown that these two alleles constitute a polymorphism. The results from experimental populations, in which these two alleles were segregating, were consistent with the hypothesis that the polymorphism was maintained by heterozygote superiority. This was confirmed by studies on various components of fitness of flies homozygous and heterozygous for $\underline{\beta_D}$ and $\underline{\beta_E}$. Heterozygote superiority was not found in all traits studied, the main difference between heterozygotes and homozygotes being in the number of fertile eggs laid.

There was some discrepancy between the selective values of the homozygotes, relative to that of the heterozygotes taken as unity, found in the experimental populations and those calculated from the fitness studies. In each case the selective values of the two homozygotes were approximately equal, but those from the fitness studies were rather <u>smaller</u> than those calculated from the approach to equilibrium in the experimental populations. The fitness studies were carried out under ideal conditions, with competition at a minimum, and these conditions do not represent those in the experimental populations where competition for food is great during all stages of the life cycle. Other workers have shown that differences between heterozygotes and homozygotes are minimised under ideal conditions, as the homozygotes are not so strongly selected against when competition is at a minimum. Thus the differing conditions in the two studies probably does not account for the discrepancy between the selective values.

In the fitness studies, all the components of fitness, with the exception of competitive mating ability in males, were measured in cultures containing only one of the genotypes found in the experimental populations. The assumption is made that any differences found in these studies reflect those that exist between genotypes when competing in the population. This ignores the effect of the frequency of other genotypes, present in the population at a particular time, on the fitness of a particular genotype. In an experiment in which the effect of genotype frequency on the viability from first larval instar stage to eclosion was studied, it was shown that the frequencies of genotypes present had an effect on this component of fitness. The effects found do not account for the discrepancy between the selective values in the experimental populations and those from the fitness studies, but the effect of genotype frequency on the other components of fitness may be responsible. This is indicated by the finding that, in the bottle population begun with a high frequency of $\underline{p_D}$, the changes in gene frequency over the first few generations were those expected from the selective values calculated from the fitness studies, while those in the bottle population, in which the initial frequency of $\underline{p_D}$ was low, were smaller than expected on the basis of the fitness studies.

In any population, factors not amenable to study, such as the ability of females to find suitable egg laying sites, will also affect total fitness, defined as the ability of carriers of a particular genotype to transmit their genes to the next generation.

In recent years the existence of increasing numbers of polymorphisms has been demonstrated, in natural and experimental populations of many species. The majority of these are protein polymorphisms. It seems that, whenever a new technique is applied, polymorphisms are revealed. To date, most of the work in this field has been concerned with the discovery and characterisation of polymorphisms. However, two estimates of the amount of heterogeneity present in natural populations have been made. Lewontin and Hubby (1966) examined a number of proteins in Drosophila pseudo obscura and their results indicated that the average population was polymorphic at 30 per cent of all loci. The estimate made by Harris (1966), using enzymes found in human blood, was similar to this. Of the ten enzymes examined, two definitely constituted genetic polymorphisms and a third was at least phenotypically polymorphic. A number of other workers have demonstrated that natural populations of Drosophila are highly polymorphic at the biochemical level, but estimates of the proportion of polymorphic gene loci could not be made in these cases because of ambiguity in the data.

Thus large amounts of genetic heterogeneity have been shown to exist in natural populations. One of the dilemmas in the field of quantitative genetics at the present time is the method by which this large amount of variation is maintained. The study of polymorphisms has shown that no single mechanism is responsible.

Some polymorphisms have been shown to be maintained by assortative mating (c.f. Sheppard, 1952), while others appear to be maintained by a type of gene frequency dependent selection (c.f. Speiss, 1957; Kojima and Yarborough, 1967). The majority of polymorphisms studied appear to be maintained by some type of heterozygote superiority, this being demonstrated directly by examination of components of fitness or indirectly from population studies. To date, however, most protein polymorphisms have merely been characterised and the mechanism by which these variants are maintained in the population is not known. It is apparent, however, from the work of Stone et al (1968) that some of these polymorphisms must be associated with fitness. These workers investigated enzyme variations in wild populations of two Drosophila species from South Pacific islands. The adaptive significance of the enzyme systems studied was not known, but the analysis of the majority allele frequencies indicated that the same alleles were at a high frequency in most enzyme systems in all populations, but there were variations of these allele frequencies which were correlated with the degree of genetic diversity among islands. This would indicate that some type of selection is involved in the maintenance of these alleles.

Lewontin and Hubby (1966) argue that the existence of a large number of polymorphisms, each maintained by heterozygote superiority, will result in a very large genetic load on the population. If the number of polymorphic loci in the population was 10^3 and selection at each locus reduced population fitness to 98 per cent of the maximum, the reproductive potential of the population would be only 10^{-9} of the maximum. Even in a species as fecund as Drosophila this would be an intolerable load.

Genetic load is defined as "the amount by which the average fitness in the population is lower than that of an individual with the optimal genotypic constitution". It is questionable how meaningful this concept of the genetic load is. The individual with the optimal genotype may be an ideal which is never achieved in natural populations and fitness may be at a maximum in a population with a number of polymorphic loci maintained by heterozygote superiority. A similar idea was put forward by Sved, Reed and Bodmer (1967). They stated that individuals with the optimum genotype are important in determining the genetic load as usually defined. However, in their model, the selective advantage at a single locus was determined by the advantage averaged over all genotypes at other loci and individuals with the optimum genotype are sufficiently rare in the population to play little part in determining this. This being so, it may be possible for a large number of polymorphisms, maintained by selection, to exist in a population. Estimates of inbreeding depression from Drosophila suggest that the number of selectively balanced polymorphisms with 1 per cent heterozygote superiority could not be greater than 10^3. King (1967) reached a similar conclusion using a model in which selection operated by culling that proportion of the population with the worst combination of genetic, non-genetic and stochastic factors affecting survival. In this model the effects of most of these factors was assumed to be additive and the values of the sums of effects normally distributed in the population.

The selective disadvantages of the homozygotes in the above models are much smaller than those found in the polymorphism controlled by the two alleles at the *p* locus. The relative adaptive values of homozygotes found in studies on other visible polymorphisms were also lower than those postulated in the above models. It is obvious that the amount of variation found in natural populations cannot be maintained by heterozygote superiority if this superiority is of the order of magnitude found in the studies on visible polymorphisms.

The estimates of the amount of variation in populations made by Lewontin et al and Harris were based on variation at the biochemical level. The number of biochemical polymorphisms found in populations is large compared to the number of visible polymorphisms. This may be due to lack of sensitivity in the methods used in the detection of the latter or it may reflect the level of biological integration at which the polymorphisms are detected. Waddington (1942) suggested that the genotype was buffered in such a way that a series of developmental pathways would give a standardised genotype. Thus the population may be able to support a larger amount of variation at the biochemical level because some of this variation is not reflected at higher levels of biological integration.

When calculating the genetic load due to a large number of loci being maintained in the population by heterozygote superiority, the assumption is made that the contributions of these loci to the total genetic load are additive. When considering the amount of variation at the biochemical level, it is probable that this assumption is not valid as it is unlikely that the proteins involved have a direct effect on fitness. As a result of interactions at higher levels of biological integration, the genetic load estimated on the assumption of additive effects may be greater than that existing in the population.

It is also possible that not all the variation present in a population at a particular time is being actively maintained by selection. Some variation may be the result of selection at some previous time and, because of changes in the environment, is no longer selected for. In large populations some variation may not be under selection either at present or at any previous time, but, if alleles are maintained at an appreciable frequency, it is likely that selection was involved at some point.

In addition to the two alleles at the *p* locus, which have been shown to constitute a polymorphism, a number of other genes affecting pigmentation of the sixth abdominal tergite have been found. Genes with an effect on pigmentation of this tergite were located on the second, third and fourth chromosomes. The effect of the X chromosome was not examined. The reason why the effects on pigmentation were specific to the sixth tergite was not clear.

The dominant gene at the *p* locus, which removes pigment from the sixth tergite, was epistatic to the other genes producing pigment in this tergite, indicating that the effect of this locus may be at some earlier step in the pathway leading to melanin production than those of the other loci. The effect of p_D did not appear to be reversible by temperature.

Waddington (1941) showed that two processes were involved in the laying down of pigment in the cuticle, firstly a deposition of brown pigment beginning 72 hours after pupation and secondly a deposition of black pigment a few hours after emergence. The genes affecting pigmentation stuidied in this thesis are associated with the amount of black pigment laid down in the cuticle. Whether they also affect the amount of brown pigment produced is not apparent. As an effect was found to be acting at approximately that time in development at which the brown pigment is thought to be laid down, it is possible that they may. The two processes leading to the formation of brown and black pigment are certainly related, both pigments being forms of melanin produced from the same substrate, tyrosine, and may be identical. The difference in colour of the two pigments may be due to their state of oxidation as it has been shown that, with ascorbic acid or hydrosulphite, melanin can be reduced from a black to a tan form.

In some insects colour pattern is determined by localisation of the substrate rather than of the enzyme. The genes studied here may affect the substrate in such a way that its movement is inhibited by those genes producing increased amounts of pigment in the sixth abdominal tergite, and accelerated by those decreasing the amount of pigment in this tergite. This would seem more likely than that they affect an enzyme in the pathway, as the effect is so localised.

The genes at the β and γ loci appear to have correlated effects on bristle number, β_D, when homozygous, increasing the sternopleural bristle score and γ_D increasing the number of bristles at the sternopleural, ocellar and abdominal sites compared to the number in flies homozygous for β_R and γ_R (c.f. Table 1). Whether these effects are due to pleiotropy or close linkage is not known. In each case, a very small region of the chromosome was involved. Special methods such as the use of diplo-X triploid females, would be necessary to distinguish between pleitropy and close linkage in the case of the fourth chromosome, as crossing over in this chromosome is a very rare event. This may only be a function of size, Sturtevant (1951) having estimated that the map length of the fourth chromosome may be as small as 0.05 cross over units, or some special mechanism suppressing crossing over may be involved. All fourth chromosomes containing γ_D that have been analysed have the same effects on bristle scores. Thus the correlated bristle effects, at sternopleural, ocellar and abdominal sites, are probably due to one gene.

If the effects on bristle number and colour pattern are pleiotropic effects of single genes, no obvious relationship between these two characters is apparent. The studies on the effect of temperature on pigmentation have shown that some effect was acting during the pupal stage, between 168 and 192 hours after the eggs were laid. Bristle formation and pigmentation are known to occur just prior to this. The relationship between bristle number and pigmentation is further complicated by the fact that sternopleural bristle number is increased by a gene $\underline{\beta_D}$ causing a decrease in the amount of pigment in the sixth abdominal tergite and also by a gene $\underline{\gamma_D}$ which increases the amount of pigment in this tergite. The problem cannot be resolved until more is known about the nature of the developmental processes involved in determining bristle number and the amount of melanin laid down in the cuticle.

The recessive allele at the $\underline{\gamma}$ locus much reduces fitness when homozygous, as well as reducing bristle number at the sternopleural, ocellar and abdominal sites. Whether this allele has a direct effect on fitness or whether this effect is due to genes closely linked to it is not known.

This raises the problem discussed earlier in connection with the amount of variation found in natural populations and the maintenance of this variation, namely the developmental processes involved in producing this variation and the relationship of these processes to the fitness of the organism as a whole. The properties of the system observed necessarily reflect the biological organisation of this system, but which properties are present as a result of pleiotropic action and which as a result of direct selection is usually unclear. A great deal of work must be done on the organisation of biological systems at the genetic, biochemical, physiological and morphological levels, and the relationship between the various levels of biological integration, before this can be resolved. As more variation is revealed, more material becomes available for use in studies on organisation.

SUMMARY

The work in this thesis is concerned with a number of loci affecting the pigmentation pattern of the sixth and seventh abdominal tergites in *Drosophila melanogaster*. During an investigation of lines selected for sternopleural bristle score, two loci had been found with an effect on pigmentation pattern. These two loci were identified and located, one, termed the *p* locus, at -1 centimorgans on the third chromosome and the other, termed the *γ* locus, on the fourth chromosome. The effects of these loci on pigmentation were completely limited to females.

By substituting second and third chromosomes from various selected lines into a line containing the recessive alleles at the *p* and *γ* loci, at least two other factors affecting pigmentation pattern were found. One of these factors was located on the second chromosome, but its expression was too variable to allow precise location, and the other on the third chromosome, probably at the ebony locus, or closely linked to it.

The two alleles at the *p* locus constitute a polymorphism. The approach to equilibrium was observed in bottle and cage populations begun with high and low initial frequencies of the dominant allele. Initial frequency did not appear to have any effect on the equilibrium point. Preliminary experiments indicated that the polymorphism was maintained by heterozygote superiority. A number of components of fitness were studied in homozygotes and heterozygotes, and heterozygote superiority was demonstrated in the viability from egg stage to eclosion, female fecundity and

longevity. The selective values of homozygotes, compared to that of the heterozygote taken as unity, consistent with the approach to equilibrium in the experimental populations, were larger, 0.68 for each homozygote, than those indicated from the fitness studies, 0.50 for each homozygote. The reasons for this discrepancy are discussed. A small experiment indicated that genotype frequency had some effect on viability, but no general trend was apparent.

The recessive allele at the *γ* locus, when homozygous, was shown to reduce viability, male mating ability and fecundity of females. The reduction in fitness was greater in males than in females.

The effects of an increase and a decrease in temperature on the expression of the alleles at the *p* and *γ* loci was studied by raising flies at 17°C and 30°C. In general, the amount of pigment in the sixth abdominal tergite was decreased at the higher temperature and increased at the lower one. Two critical periods for the temperature changes, resulting in an increase in the amount of pigment, were found, one during the eighth day of development and a second just after emergence.

The problem of the maintenance of the large amount of variation, shown to exist in natural populations, is discussed.

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ABSTRACT OF THESIS

ame of Candidate JANICE ROGERS
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egree Ph.D. Date November, 1968.
le of Thesis STUDIES ON BODY COLOUR POLYMORPHISM IN DROSOPHILA MELANOGASTER.

The work in this thesis is concerned with a number of loci affecting the pigmentation pattern of the sixth and seventh abdominal tergites in Drosophila melanogaster. During an investigation of lines selected for sternopleural bristle score, two loci had been found with an effect on pigmentation pattern. These two loci were identified and located, one, termed the p locus, at -1 centimorgans on the third chromosome and the other, termed the γ locus, on the fourth chromosome. The effects of these loci on pigmentation were completely limited to females.

By substituting second and third chromosomes from various selected lines into a line containing the recessive alleles at the p and γ loci, at least two other factors affecting pigmentation pattern were found. One of these factors was located on the second chromosome, but its expression was too variable to allow precise location, and the other on the third chromosome, probably at the ebony locus or closely linked to it.

The two alleles at the p locus constitute a polymorphism. The approach to equilibrium was observed in bottle and cage populations begun with high and low initial frequencies of the dominant allele. Initial frequency did not appear to have any effect on the equilibrium point. Preliminary experiments indicated that the polymorphism was maintained by heterozygote superiority. A number of components of fitnes were studied in homozygotes and heterozygotes, and heterozygote superiori was demonstrated in the viability from egg stage to eclosion, female fecundity and longevity. The selective values of homozygotes, compared to that of the heterozygote taken as unity, consistent with the approach to equilibrium in the experimental populations, were larger, 0.68 for

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each homozygote, than those indicated from the fitness studies, 0.50 for each homozygote. The reasons for this descrepancy are discussed. A small experiment indicated that genotype frequency had some effect on viability, but no general trend was apparent.

The recessive allele at the *γ* locus, when homozygous, was shown to reduce viability, male mating ability and fecundity of females. The reduction in fitness was greater in males than in females.

The effect of an increase and a decrease in temperature on the expression of the alleles at the *p* and *γ* loci was studied by raising flies at 17°C and 30°C. In general, the amount of pigment in the sixth abdominal tergite was decreased at the higher temperature and increased at the lower one. Two critical periods for the temperature changes, resulting in an increase in the amount of pigment, were found, one during the eighth day of development and a second just after emergence.

The problem of the maintenance of the large amount of variation, shown to exist in natural populations, is discussed.