POLYMORPHISM AT THE EBONY LOCUS OF *DROSOPHILA* *MELANOGASTER* AND POPULATION FITNESS

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CARSON (1957) suggested that the biomass (wet weight of adults) produced per unit time may be used as a measure of the fitness of a population. Various workers (e.g. CARSON 1958; BEARDMORE, DOBZHANSKY, and PAVLOVSKY 1960; DOBZHANSKY and PAVLOVSKY 1961) have shown the population size, and the number and weight of adults emerging in a given time to be greater in populations polymorphic for a number of loci or for chromosomal inversions, than in corresponding monomorphic populations. By this measure of fitness, polymorphic populations had higher fitness than monomorphic populations.

For several recessive genes in *D. melanogaster*, apparently stable equilibrium gene frequencies have been found in experimental populations segregating for the recessive and its wild-type allele, giving rise to a single locus polymorphism (e.g. thread (*th*), HEXTER 1955; sepia (*se*), cardinal (*cd*), short vein (*shv*), brown (*bw*), raised (*rsd*), RASMUSSEN 1958; sepia (*se*), spineless (*ss*), rough (*ro*), CARSON 1958; cinnabar (*cn*^s), FRYDENBERG and SICK 1960, 1962; and ebony (*e*), see below). Such cases provide convenient material for investigation of heterosis and polymorphism. The most widely studied of these loci has been ebony, for which an apparently stable polymorphism has been reported for various alleles (L'HÉRITIER and TEISSIER 1937; KALMUS 1945; TEISSIER 1947; CARSON 1958; RASMUSSEN 1958; ZÜRCHER 1962, 1963; and MOREE 1963).

In Experiments 1 and 2 reported here, we have investigated further the apparent stability of an ebony polymorphism, and the effect of this polymorphism on population numbers, number of flies emerging from a medium jar, rate of development, and sex ratio.

The selective changes in the frequencies of genotypes in populations will be affected by any interactions between the genotypes affecting viability or other fitness components. BEARDMORE (1963) found that the increased fitness of polymorphic populations of *D. pseudoobscura* was in part due to favourable interactions between larvae of different genotypes affecting their viability. Similar interactions have been found in *D. melanogaster* (LEWONTIN 1955) and in *D. busckii* (LEWONTIN and MATSUO 1963). WEISBROT (1964) suggested these interactions were due to some accumulated waste product of the larvae. The possibility of such interactions affecting viability at the ebony locus, and contributing to the development of polymorphism, was also investigated (Experiment 3).

MATERIALS AND METHODS

The population cages used (a modification of those of BARKER 1960), were made of galvanised iron with overall dimensions $15 \times 11 \times 8$ inches. Medium jars (100 ml beakers, 2 inch diameter) were placed in nine metal rings on the floor of the cage. Jars were replaced three times each week (Monday, Wednesday, and Friday), so that a jar remained in a cage for 21 days. Each jar contained 30 ml of a dead-yeast fortified medium. In Experiment 2, medium F of CLARINGBOLD and BARKER (1961) was used, while in Experiment 1 we used a modification of this medium with 7 g of agar and 700 ml of water. All jars were seeded with 3 ml of live yeast suspension containing 1.5 g compressed yeast. Under these conditions, the generation interval of *Drosophila melanogaster* is about 23 days (BARKER 1962).

To census a population, all adults were removed under low vacuum pressure. A random sample of approximately 1000 individuals was taken, censused and weighted. A comparison of the weight of the sample with that of the total population provided an estimate of the population size.

The populations were maintained at $25 \pm 0.5^\circ\text{C}$. Experiment 1 was commenced in a room lit continuously, but after six months the cages were transferred to a room lit for 12 hours daily (6 AM to 6 PM). Experiment 2 was carried out under the latter conditions.

Strains used: Oregon-R-C—Wild-type stock of *D. melanogaster* maintained since September, 1958 in a population cage averaging 4,000 to 5,000 adults. e^{11} (ebony body colour)—Isogenised with Oregon-R-C by 27 generations of backcrossing, mass-mated since then for approximately 40 generations.

Methods used for Experiment 3 are detailed separately.

RESULTS

Experiment 1. Populations of three genetic compositions (homozygous wild type, homozygous ebony, and competing wild-type/ebony—here called mixed), were compared for population size and sex ratio, and the change in frequency of ebony was observed in mixed populations. Cages A-1 and A-2 were initiated on June 18, 1962 with 200 heterozygous flies ($+/e^{11}$) from a cross $+/+$ female $\times e^{11}/e^{11}$ male. Cages A-9 and A-10 were initiated with 200 ebony flies, and A-11 and A-12 with 200 wild-type flies on October 10, 1962. Samples of adults from the mixed cages were censused occasionally during the first four months after initiation, to measure the frequency of ebony. Adults in all cages were censused on February 25, 1963 (36 weeks after initiation of the mixed cages), and transferred to fresh cages, the medium jars not being transferred. Cages were then censused weekly at first, and later less frequently.

Change in gene frequency: The change in frequency of ebony homozygotes from three weeks after initiation is shown in Figure 1. The frequency decreased rapidly over the first few months. However, from week 12 to week 46, the populations remained in apparent stable polymorphism, with ebony homozygote frequency fluctuating from 1 to 3%. From week 46, the frequency declined to under 1% in week 59. In week 72, following a drastic drop in population size due to mould contamination, the percentages of ebony homozygotes in A-1 and A-2 were zero and 0.83. The populations were transferred to clean cages, and censused in weeks 88, 117, and 143, when the percentages of ebony homozygotes were 0.96 and 0.77, 0.65 and 0.32, and 0.31 and zero. At week 150, ebony gene frequency was estimated from an egg sample. No ebony homozygotes were found in approximately 2000 flies from either cage. Samples of these flies (144 from

FIGURE 1.—Change in frequency of ebony homozygotes in mixed cages.

A-1, and 136 from A-2) were progeny tested to determine their genotypes. Only three heterozygotes were observed in each sample, so that the ebony gene is apparently being slowly eliminated.

Population numbers: The estimated total numbers of adults in the cages at censuses from February 25, 1963, are shown in Figure 2. Population numbers increased to a maximum in about four weeks, and then declined. Analysis of population numbers was done as a replicated factorial (3 genetic compositions $\times$

FIGURE 2.—Estimated total numbers of adults in the cages from the time of transfer to fresh cages on February 25, 1963.

8 weeks), omitting results from the first two censuses. These included only survivors of the transferred adult populations at the first census, and these together with their first emerging progeny at the second census.

The mean number of adults in the mixed populations (4002), was higher than in ebony (3592), and wild type (3476), but the differences were not significant. Variation among weeks was highly significant ($F = 6.36$, $P < 0.001$).

Experiment 2. Samples of adults taken from cages A-9 (ebony) and A-11 (wild type) were placed in culture bottles (ten pairs per bottle) for three days, and then returned to the cages. Virgin progeny were collected, and used to initiate the cages when 2 to 5 days old. Cages B-1 and B-2 were initiated with 2000 wild-type flies, B-3 and B-4 with 2000 ebony flies, and B-5 and B-6 with 1500 ebony and 500 wild-type flies. Equal numbers of each sex were used in all cages.

Estimates of the changes in frequency of ebony homozygotes in the mixed cages, and data on population numbers and sex-ratio in all cages were obtained using three census procedures: (1) *Adult populations.* These were censused weekly by the sampling technique described under MATERIALS and METHODS. (2) *Egg samples.* During the 24 hours prior to an adult census, a medium jar was left in each cage to obtain an egg sample. Sections of the medium surface of each sample were transferred into four culture bottles containing medium F. All emerging adults were censused for sex and phenotype. After 13 weeks, egg samples were taken only from the mixed populations, and were put into eight culture bottles. Samples were taken to give approximately 200 emerging adults per bottle, which thus developed under uncrowded conditions. In mixed populations, differences in the frequency of ebony homozygotes in an adult population census and in the concomitant egg sample would indicate either selective mating or differential fecundity of the genotypes.

(3) *Medium jar emergences.* The jars placed in the cages each Friday (two days after adult census) were emptied of adults four days later and covered with nylon gauze. All emerging adults from these were censused daily for the first week of emergence, and then every second day, except when large numbers were still emerging. They were added to the cage population after census. These flies, in contrast to those from egg samples, therefore develop under the typically heavily crowded cage jar conditions. Any differences in ebony homozygote frequency between these and the concomitant egg sample would largely reflect differential viability of genotypes during development in the cage environment.

Wild-type contaminants were observed in cage B-4 after nine weeks. Removing these from the medium jar emergences and at the weekly adult censuses kept them at a low frequency ($< 5\%$).

Change in frequency of ebony: The changes in frequency of ebony flies in cages B-5 and B-6 as estimated from the adult population, medium jar emergences, and egg samples are shown in Figure 3. As the cages were not initiated with genotypes in Hardy-Weinberg equilibrium, there were wide fluctuations in frequency as estimated by the three census procedures over the first few weeks. However, the frequency of ebony homozygotes decreased rapidly to about 9% at eight weeks, after which the decline was more gradual to about 3% at week 23. In two further adult censuses, the percentages were 1.10 and 0.93 (week 34), and 0.20 and 0.56 (week 47) for B-5 and B-6 respectively. In these cages, there is no evidence of stable polymorphism, and the ebony gene is apparently being steadily eliminated. Linear regression equations for decrease in percentage of ebony homozygotes from week 8 to week 23 were calculated. All regression coefficients were significant, but there were no significant differences between the equations either for

FIGURE 3.—Change in frequency of ebony homozygotes in mixed cages as estimated from the adult population, medium jar emergences, and egg samples.

the different census procedures or for the two cages. Within the accuracy of the experiment, the frequency of ebony could be estimated equally well by any of the three census procedures. For those weeks of weeks 8 to 23 where all three census procedures were used, the mean percentages of ebony homozygotes may be compared to provide some indication of the selective processes (Table 1). The percentage in egg samples is about equal to or greater than that in the concomitant adult population. ELENs (1957) showed that the sexual activity of ebony males was less than that of wild type. However, heterozygotes show mating superiority (ELENs 1958; JACOBS 1961) and superior fecundity (MOREE 1962). In our populations, this heterozygote superiority apparently compensates or even overcompensates for the effect of selective mating acting against ebony. The per-

TABLE 1

Average percentages of ebony homozygotes in the three census procedures over weeks 8 to 23

	Percentage e^{11}/e^{11}	
	B-5	B-6
Adult populations	6.12	5.46
Egg samples	6.11	6.59
Medium jar emergences	5.40	4.95

centage of ebony in the medium jar emergences is less than that in either the adult population or egg sample. This would be expected, as MOREE and KING (1961) found the relative viability of e^{11}/e^{11} to be less than those of $+/+$ and $+/e^{11}$, and further that it was consistently decreased by increased larval crowding.

Population numbers: The estimated numbers of adults in the cages are shown in Figure 4. In the analysis of these numbers after the second census, the difference among weekly means was significant ($F = 3.27$, $P < 0.01$). Population numbers increased to about the sixth week after initiation, and then fluctuated considerably. The difference among genetic compositions was highly significant ($F = 17.89$, $P < 0.001$), owing to the smaller numbers in the ebony cages (mean 3714) than in wild type (5492) and mixed (5904), the difference between these two not being significant.

Number of emergences from a medium jar: The numbers of medium jar emergences are shown in Figure 5. Very few flies (200 to 300) emerged from the jars placed in the cages two days after initiation (week 0), probably because of the extremely large number of eggs that would have been laid with 1000 healthy females present. Results from this week were omitted in the analysis of variance. The difference between replicates within genetic compositions was significant when tested against the residual variance ($F = 4.54$, $P < 0.01$), and on using the replicate variance as error variance, the difference among genetic compositions was not significant. The mean numbers emerging were 1825 for

FIGURE 4.—The estimated numbers of adults in the cages. Wild type: B-1 $\bigcirc$ --- $\bigcirc$, B-2 $\times$ --- $\times$. Ebony: B-3 $\bigcirc$ — $\bigcirc$, B-4 $\times$ — $\times$. Mixed: B-5 $\bigcirc$ — $\bigcirc$, B-6 $\times$ — $\times$.

FIGURE 5.—The numbers of medium jar emergences. Designation of cages as in Figure 4.

mixed, 1430 for wild-type, and 942 for ebony. The difference among weeks also was not significant.

Rate of development: The time from placing a medium jar in a cage until the mean time of emergence of adults was measured for jars placed in the cages from weeks 4 to 16 inclusive. The mean developmental times and the average within jar variances for each cage are shown in Table 2. For all cages, the within jar variance fluctuated widely from week to week, and these variances were found to be heterogeneous (Bartlett's test). Analysis of variance, unweighted for heterogeneity, showed a highly significant difference between replicates within genetic compositions ($F = 13.94$, $P < 0.001$), and again using the replicate variance as error, the difference among genetic compositions was not significant. The mean time of development was 11.44 days for mixed, 12.71 days for wild type, and 12.98 for ebony populations.

Sex ratio: The percentages of females in the total number counted and the mean of individual percentages of weekly totals for the adult population in

TABLE 2

Mean developmental times and their average within jar variances for medium jars placed in cages from weeks 4 to 16 inclusive

	Wild type		ebony		Mixed	
	B-1	B-2	B-3	B-4	B-5	B-6
Mean time (days)	13.47	11.96	14.09	11.87	11.85	11.03
Variance	3.20	1.78	5.80	1.88	2.52	1.58

Experiment 1, and for the adult population, medium jar emergences, and egg samples in Experiment 2, are shown in Table 3. In Experiment 1, there was an excess of males in both pure wild-type and ebony cages, which was significant for all except a few weeks when the numbers were increasing. In the mixed cages, there was a large excess of females in the first two censuses, after which the sex ratio did not differ significantly from unity.

In Experiment 2, there was a significant excess of males in all cages for both the adult population (except for B-1), and for the medium jar emergences (except for B-2). There was no apparent relationship between sex ratio at emergence and in the adult population. In the egg samples, raised under optimum conditions, sex ratio did not differ significantly from unity. Regressions of sex ratio on weeks and population numbers were estimated for both adults and medium jar emergences, but none differed significantly from zero.

Experiment 3.

Newly hatched first instar larvae (collected 22 to 24 hours after a one-hour egg sampling period), were collected and put in 3×1 inch vials containing 4 ml of ordinary cornmeal medium (5 g agar, 740 ml water, 40 ml treacle, 100 g cornmeal, 0.8 g Nipagen-M) to which a small amount (1.2 g) of dead yeast was added, and with no live yeast seeding. Densities used were 4, 16, 64, 128, and 256 larvae per vial. At each density, vials were established with larvae of the following genotypes: $+/+$ homozygotes, $+/e^{11}$ heterozygotes, e^{11}/e^{11} homozygotes, and with the three genotypes in Hardy-Weinberg frequencies with ebony gene frequencies of 25%, 50% and 75%. At a density of four larvae, the 25% and 75% ebony gene frequency

TABLE 3

Percentage of females in samples of adults in Experiment 1, and in samples of adults, medium jar emergences, and egg samples in Experiment 2

Experiment 1	Wild type		ebony		Mixed	
	A-11	A-12	A-9	A-10	A-1	A-2
<i>Adults</i>						
Percentage of total number counted	41.74	44.75	38.22	42.13	52.91	52.61
$\chi^2_{(1)}$	481**	178**	989**	472**	52**	66**
Mean of percentages	42.89	42.05	38.12	42.32	51.62	51.52
Experiment 2	B-1		B-3		B-5	
	B-1	B-2	B-3	B-4	B-5	B-6
<i>Adults</i>						
Percentage of total number counted	45.27	49.26	43.13	48.60	48.26	48.22
$\chi^2_{(1)}$	214**	4.4*	365**	11.2**	32.8**	32.2**
Mean of percentages	45.25	48.60	43.34	47.09	48.45	48.51
<i>Medium jar emergences</i>						
Percentage of total number counted	49.57	48.23	47.44	47.08	48.28	47.08
$\chi^2_{(1)}$	1.5	32.9**	37.3**	49.9**	61.7**	115**
Mean of percentages	49.74	48.20	47.15	46.84	48.20	46.85
<i>Egg samples</i>						
Percentage of total number counted	49.76	50.01	50.65	50.25	50.34	49.90
$\chi^2_{(1)}$	0.17	0.04	1.05	0.14	0.86	0.06
Mean of percentages	50.47	49.71	50.68	50.54	50.77	49.49

* $P < 0.05$. ** $P < 0.001$.

FIGURE 6.—Effect of larval density on percentage viability.

compositions are, of course, not possible. Ten replicates were set up (at weekly intervals of three, three and four) and for each genetic composition in each replicate 1 vial with 256, 1 with 128, 2 with 64, 4 with 16, and 16 with 4 larvae per vial were used. All emerging adults were censused daily, allowing calculation of the viability of each genotype, as the percentage that adults emerging were of the number of larvae set up.

The percentage viability for the various treatments is shown in Figure 6. For all densities, there were no significant differences between the genotypes in viability in either pure or mixed cultures. Thus, for the mixed culture results in Figure 6, only the overall percentage viability is shown, and not those for the separate genotypes. For all genotypes, there was little change in viability from 4 to 64 larvae per vial, but at the higher densities, there was an appreciable decline in viability. However, the depression in viability was not sufficient to detect differences between genotypes affecting viability or the presence of interactions between competing genotypes.

DISCUSSION

At the time the study of population numbers in Experiment 1 was done, ebony appeared to be in equilibrium with its wild-type allele, with ebony homozygotes at a frequency of about 2%. However, this apparent polymorphism is unstable, as later censuses indicate that ebony is being slowly eliminated. Similar results were obtained by FRYDENBERG (1964), except that the apparent equilibrium in his populations was at a higher frequency (about 10%) of ebony homozygotes. In other long-term studies of competing ebony and wild-type genotypes, TEISSIER (1947) found that, in time, an apparent equilibrium gene frequency changed to a new lower level, while POLIVANOV (1964) showed changes in the relative fit-

nesses of the three genotypes over about 40 generations of natural selection, but with no evidence of overdominance at any stage. Similarly, in Experiment 2, there was no indication of the development of polymorphism, as ebony homozygote frequency decreased steadily to an average of less than 0.5% in week 47, when the cages were terminated.

These results do not agree with previous studies, where apparently stable polymorphism has been reported for various ebony alleles (L'HÉRITIER and TEISSIER 1937; KALMUS 1945; TEISSIER 1947; CARSON 1958; RASMUSSEN 1958; ZÜRCHER 1962, 1963; MOREE 1963.) Also, studies on several fitness components (ELENIS 1957, 1958; JACOBS 1960, 1961; MOREE and KING 1961; MOREE 1962; ZÜRCHER 1963) have shown heterozygote superiority. However, this need not reflect intrinsic overdominance at the ebony locus. Heterozygote superiority for one or two components of fitness need not mean superiority for overall fitness. Also, in some of these experiments, no attempt has been made to isogenise the wild-type and ebony strains, so that heterosis may result from genetic differences between the strains at other loci. Inter-population hybrids in *Drosophila* species have often shown heterosis, but this breaks down in the following generations (BRNCIC 1954; VETUKHIV 1954; WALLACE 1955). Thus, for example, the superior viability of heterozygotes produced as F_1 crosses between ebony and wild-type strains may not be maintained in populations.

Further, in laboratory population studies, such luxuriance (DOBZHANSKY 1955) is unlikely to lead even to a temporary polymorphism. However, CARSON (1958) and CANNON (1963a) have shown that the introduction of a single male taken from an F_1 cross between Oregon-R and another strain (sesro—homozygous for five chromosome-3 oligogenes) into a sesro population results in rapid, marked, and prolonged increases in population fitness, associated with maintenance of the mutant genes in apparent equilibrium. CANNON has referred to this heterosis as “a kind of captured luxuriance.” The initial heterozygosity apparently causes the initial increase in fitness and may be responsible also for the maintenance of this fitness and for the associated balanced polymorphism. SMATHERS (1961) showed this heterosis to be mainly due to heterozygosity for polygenes or unknown oligogenes, and not due to heterozygosity for the sesro oligogenes. Thus the relative fitnesses of genotypes at a single locus may depend on the effects of polygenic complexes linked with them. BODMER and PARSONS (1960, 1962) and PARSONS and BODMER (1961) argued that apparent overdominance may often result from the selection of balanced polygenic complexes. In the initial population formed from a cross of two previously isolated strains, linkage disequilibrium may allow the development of an apparent gene frequency equilibrium. In time, this may be destroyed by recombination, or alternately, permanent linkage disequilibrium may result (CANNON 1963b; CHIGUSA and MUKAI 1964), perhaps due to epistatic gene interactions (LEWONTIN and KOJIMA 1960). This could explain the decay of an apparently stable ebony polymorphism reported by FRYDENBERG (1964), as the parental strains were not isogenised.

DAWOOD and STRICKBERGER (1964) have argued that apparent overdominance at the ebony locus may depend on the relative heterozygosity of the strains used,

even where the ebony and wild-type strains have been isogenised. These strains have generally been laboratory populations that will be partially inbred, so that only few wild-type isoalleles may have been segregating. To the extent that superior fitness depends on heterozygosity at many loci, then the poor performance of wild-type homozygotes may have been due to their relatively high degree of homozygosity, as compared with the $+/e$ heterozygotes that would be at least heterozygous for genes near the ebony locus. This possibility is supported by their results on viability studies in single generation cultures, and by the population studies of POLIVANOV (1964), where the decline in ebony gene frequency was more rapid when the initial wild-type population comprised a mixture of a number of strains, as compared with just one strain. This explanation may apply to our experiments, where only one wild-type strain was used, but the parental strains had been isogenised by 27 generations of backcrossing. The results in Table 1, although giving only crude estimates of the selective processes, suggest that any heterozygote superiority in certain fitness components such as mating ability and fecundity is completely outweighed by the reduced viability of ebony homozygotes. However, this refers to Experiment 2, and our experiments had two different outcomes. In Experiment 1, there was an apparent polymorphism that eventually decayed, while in Experiment 2, there was no evidence of even such a transient polymorphism. This difference, and the varied results of different studies may be due to differences in experimental conditions. Different media were used in the two experiments, and BARKER (1963) has shown significant effects of media on the relative fitness of the Oregon-R-C wild-type strain used in these experiments.

Experiment 3 was designed to determine the importance of viability as a component of fitness for this locus. However, even at the highest larval density and consequent lowest viability, there were no significant differences among genotypes nor interactions between them. MOREE and KING (1961) found the relative viability of ebony decreased as crowding increased, and found evidence of heterosis, but they did not use isogenic parental strains. DAWOOD and STRICKBERGER (1964) also found that the viability of ebony declined with increased crowding, but found evidence of heterosis for viability only when both crowding and the degree of homozygosity of the wild-type strain were high.

Not only has the polymorphism for ebony proved unstable, but there is no evidence of increased fitness in the mixed populations. In Experiment 1, when adult censuses were done during the period of apparent stability of ebony frequency, differences among genetic compositions were not significant, although mean population numbers were higher for the mixed cages. In Experiment 2, differences among genetic compositions in adult population number were significant, but the mixed and wild-type populations did not differ. For medium jar emergences, the numbers in mixed populations were larger than in pure populations, but not significantly so because of the large variation between replicate cages within genotypes. Similarly, developmental time was shorter in the mixed cages, but not significantly so.

Although the sex ratio data do not form an integral part of this study, they are

of interest in that this parameter appears to have been generally neglected in previous population studies. THOMSON (1961) obtained sex ratios differing significantly from unity in either direction, among the adults of several populations, where white (w) and satsuma (w^{sat}) were competing in population cages. However, under cage conditions in our experiments, females consistently had a lower survival rate than males in the preadult stage, and often in the adult stage as well. The percentage of females therefore was less in the medium jar emergences (high larval density) than in the egg samples (relatively uncrowded). This is the reverse of the results of SOKOLOFF (1955), who found that, for *D. persimilis* and *D. pseudoobscura*, the percentage of females increased with increased larval density, while BARKER (1965) extended this finding to population cage conditions for *D. pseudoobscura*. ANDERSEN (1961) stated that, with increasing crowding, no decrease in percentage of females at emergence had been found in species with the male heterogametic. This is in accordance with the tendency of the homogametic sex to show lower mortality, because of the effect of deleterious sex-linked recessives. The excess of males in the medium jar emergences in Experiment 2 indicates that causes other than sex-linked recessives affect sex ratio. Further, PODGER and BARKER (unpublished) found that the sex ratio at emergence varied with density for the same Oregon-R-C strain as used here. Using 2 ml of a medium similar to that used in Experiment 3, they varied larval density from 4 to 256 larvae per vial. The percentage of females declined from 49.5% at low crowding to 39.0% at 256 larvae per vial.

These differences between the two species *D. melanogaster* and *D. pseudoobscura* in the effect of larval density on sex ratio, and the possible significance of selective differences between the sexes in competition between genotypes (see BARKER 1965) would suggest that analysis of sex ratio may provide evidence on the selective changes occurring under population conditions.

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SUMMARY

The stability of a polymorphism at the ebony locus and its effect on several components of population fitness were investigated. An apparently stable equilibrium with ebony flies at a frequency of about 2% persisted for about 30 weeks. However, this equilibrium proved to be unstable, as later censuses indicated that the ebony gene would be eventually eliminated.—The polymorphism appeared to have produced a slight but nonsignificant increase in population fitness. Appreciable differences between replicate cages masked any differences between genetic compositions.—An excess of males was frequently found both in the adult population and in medium jar emergences, indicating that females had lower viability in both preadult and adult stages.—The effect of larval density

on viability was studied in both pure cultures of the three genotypes and in cultures containing all three genotypes. No differences in viability were detected between the genotypes at any of the densities studied. However, a comparison of the frequency of ebony in medium jar emergences with that in egg samples raised under optimum conditions, suggested that the viability of ebony in the cage populations was lower than that of the other two genotypes, as a consequence of crowding.

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