SELECTION COMPONENTS IN BACKGROUND REPLACEMENT LINES OF DROSOPHILA

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ABSTRACT

Selection components analysis was performed in lines of *Drosophila melanogaster* at three times during substitution backcrossing. The initial two lines were inbred isofemale lines from natural populations in California, and one had the spread wing mutation eagle. The selection components analysis revealed aspects of the genetic structure of the determinants of fitness by demonstrating changes in the marginal fitnesses of the eagle locus. Differences among backgrounds essentially disappeared by the 20th generation of backcrossing, suggesting that the previously observed differences were attributable to linkage disequilibrium. The method of bootstrapping was used as a novel means of determining statistical confidence in selection components.

THE assignment of causality of particular phenotypic attributes to particular genetic loci remains a significant problem to population geneticists. This is particularly so in the case of phenotypic attributes associated with fitness, because these attributes are fundamental in determining the evolutionary dynamics of polymorphisms. The causality problem can be split into two independent issues: linkage disequilibrium and context dependence. Despite the general lack of significant linkage disequilibrium among allozyme loci in natural populations (LANGLEY, TOBARI and KOJIMA 1974; MUKAI, WATANABE, YAMAGUCHI 1974; CHARLESWORTH *et al.* 1979), it is incorrect to suggest that linkage disequilibrium has no evolutionary consequence. Linkage disequilibrium can be critically important in determining the dynamics of newly arisen mutant alleles (ASMUSSEN and CLEGG 1981), since the initial dynamics of a newly arisen allele depend upon the phenotypic affects of the alleles with which it is associated. At the molecular level it may be particularly difficult to rule out the influence of flanking regulatory sequences in the phenotypic manifestation of a structural gene. If linkage disequilibrium is absolute, this is not a serious problem, because such tightly linked sequences will behave as a single gene. Linkage disequilibrium at the nucleotide level, however, is not always absolute (KREITMAN 1983).

Even in the absence of a structured genetic background, causality may be difficult to assign, because the expression of many genes depends on both the genetic and environmental context. Regulatory sequences are not always tightly

linked to their structural target loci (ABRAHAM and DOANE 1978; LAURIE-AHLBERG *et al.* 1982), and phenotypic expression of the structural loci in these cases clearly depends on other loci. Background effects, epistatic interactions and gene by environment interactions can never be absolutely excluded, and conclusions that these are unimportant are inductive. One cannot test the effect of an allelic substitution in all possible contexts.

A number of experimental means have been employed to sharpen the assignment of causality of fitness attributes to identified loci. Mother-offspring data can be tabulated and selection components analysis performed by sampling individuals of different ages from a natural population (CHRISTIANSEN and FRYDENBERG 1973; CHRISTIANSEN, FRYDENBERG and SIMONSEN 1977). Although linkage disequilibrium and genetic interaction may go undetected, a significant advantage of this method is that the genetic and environmental context in which the estimates are made is the natural context. Another approach is to bring many lines into the laboratory and to examine fitness attributes in contrived experiments. This method suffers the same problem as the previous method, and, although the genetic context may be close to natural, the environmental context probably is not. On the other hand, environmental control allows assessment of the sensitivity of gene expression to environmental variation. This method has been used with some success by altering the composition of the medium in a way thought to specifically address the mechanism of action of an allozyme (DEJONG and SCHARLOO 1976; HICKEY 1979; CAVENER and CLEGG 1978). Finally, the genetic background can be manipulated by intercrossing to "randomize" associations, by generating coisogenic lines (EANES 1984, CLARK and DOANE 1984), by transduction (DYKHUIZEN and HARTL 1980) or by substitution backcrossing. Although these methods are of limited efficiency, and may introduce artifacts such as hybrid dysgenesis, they have the advantage in allowing an assessment of the importance of genetic context in gene expression. If the objective is to "explain" a polymorphism, then selection components analysis in a natural context is the superior method, but, if one wants to explore the structure of the genetic determination of fitness, a more manipulative procedure is needed.

The issue is made even more complex by virtue of the fact that the phenotype of interest is multidimensional. A polymorphism may be affected by gametic selection, differential viability, sexual selection or differential fecundity. These different aspects of the phenotype may be strictly causally related to the identified locus, and hence represent pleiotropic effects, or they may be attributable to linked genes. Demonstration of pleiotropy, therefore, entails exclusion of background influence. In this paper the genetic background of lines of *Drosophila melanogaster* is modified by intercrossing and substitution backcrossing, and selection components tests are performed to address the questions outlined.

MATERIALS AND METHODS

Drosophila strains: GB8S is a strain descended from a single female caught in April 1980 by J. COYNE at the Gundlach-Bundschu winery in Napa, California. This strain is homozygous for an

allele at the eagle (*eg 3-47*) locus designated eg^{GB8S}(or *eg*), giving the flies a spread-wing phenotype. This strain was maintained in vials for 2 yr; then, six generations of brother-sister mating were performed before the beginning of the experiment. This strain had a moderate *P* cytotype, scored by gonadal dysgenesis.

Wd-4 is a strain descended from a single female caught in April 1980 by A. CLARK in Woodside, California. This strain exhibits a normal wing phenotype, and its allele at the *eg* locus is designated eg^+ or simply +. Brother-sister mating had been performed for ten generations before the beginning of the tests reported here. This strain had an *M* cytotype.

The Harwich strain was descended from two females collected by M. TRACEY, JR., in 1967 at Harwich, Massachusetts, and was kindly given to us by M. G. KIDWELL. This strain had a strong *P* cytotype and was used as a tester stock.

The Canton-S standard laboratory strain bears the *M* cytotype and was used as a tester stock in the determination of cytotypes.

Penetrance tests: With a quantitative character like wing spreading, it is crucial to show that the phenotypic distributions of the different genotypic classes do not overlap significantly. Penetrance is defined here as the fraction of flies known to be *eg/eg* that were scored as spread-wing phenotype. It was scored by performing reciprocal test crosses between known *eg* homozygotes and apparently wild phenotype flies. Additional tests were performed to assess the developmental stability of the *eg* phenotype, and no effects of temperature or crowding were detected.

Backcrossing schemes: The four different crossing schemes that were used to change the genetic backgrounds are depicted in Figure 1. The GB-MIX and Wd-MIX schemes result in genetic backgrounds bearing alleles from both initial strains, the former in a GB8S cytoplasm and the latter in a Wd-4 cytoplasm. The GB-REP scheme results in a replacement of the flanking genes of eg^+ with the GB8S background. The resulting strains are often referred to as being congenic. The Wd-REP scheme results in flies with the Wd-4 background bearing alleles eg^{GB8S} and eg^+. At each generation approximately five pairs were placed in each of 18 vials to propagate each line.

Selection components tests: Phenotypic segregations were scored among the progeny of the crosses *eg/+* × *eg/eg*, *eg/eg* × *eg/+* and *eg/+* × *eg/+* in each background at generation 0, 10 and 20 of the replacement sequence. Other components of selection were also assessed at these times by performing single-generation transition experiments. Within each background, wild phenotype females were crossed to *eg* phenotype males, yielding wild phenotype heterozygotes. These heterozygotes were crossed, the F_2 progeny were collected as virgins and aged 2–4 days and 150 pairs were mass mated in 500-ml bottles containing 42 ml of medium for 2 hr. After etherization, males were discarded and females were placed individually in vials containing sucrose-agar-yeast medium. Vials were labeled by female phenotype, and the females were removed after 4 days of egg laying. Progeny were reared under continuous illumination at 25° and were scored by wing phenotype on day 16. Paternal phenotype associated with each sibship was not known, and mating types had to be inferred from maternal and offspring phenotypes. Effects due to variation in fecundity, sexual selection and zygotic selection were assessed using a nonparametric procedure known as bootstrapping (EFRON 1979, 1981, 1982).

Population bottles: After the 20th generation of backcrossing, *eg* phenotype flies were crossed with wild phenotype flies within each background. Wild phenotype progeny were genotypically *eg/+*, and 25 pairs of these flies were used to start each population. Two replicates of each of the four background populations were followed for 12 discrete generations in 6-ounce urine analysis bottles. Adult progeny were transferred on day 0 to bottles containing fresh medium. Twenty-four hours of egg laying were allowed, and then flies were scored by phenotype and discarded. On day 14 all of the adult progeny were again transferred to fresh bottles to begin the next generation.

Cytotype tests: Since transposable elements had been implicated in the natural polymorphism of *eg* (GREEN 1982), it was important to test the cytotypes of strains. An *M* cytotype *eg* strain was chosen deliberately in order to study the relationship between hybrid dysgenesis and the population behavior of *eg*. Cytotypes were tested by reciprocally crossing the unknown strain to both Harwich and Canton-S. Progeny were reared at 29°, and adult female progeny were dissected under alcohol (SCHAEFER, KIDWELL and FAUSTO-STERLING 1979). Flies were scored as dysgenic when one or both ovaries failed to develop normally.

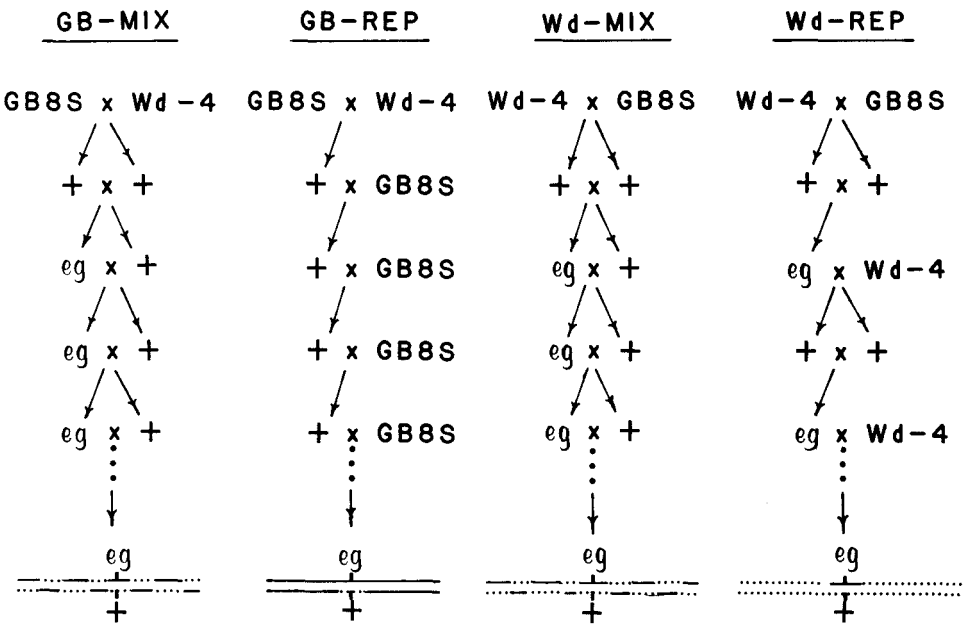

FIGURE 1.—Patterns of backcrossing and intercrossing used to generate four different genetic backgrounds. Female genotype or phenotype is given on the left of each cross. *eg* refers to the *eg* phenotype. At the bottom are diagrams of the configurations of chromosome segments in heterozygotes assuming GB8S was initially represented by a solid line and Wd-4 was represented by a dotted line. "Generation 0" tests were performed with GB8S, Wd-4 stocks and their F_1.

RESULTS

Penetrance: A total of 200 flies from the F_2 generation were tested for penetrance and, of the flies that were genetically *eg/eg*, 98.5% had spread wings. None of the flies that were genetically *eg*/+ or +/+ were misclassified as spread-wing phenotypes, demonstrating full recessiveness of the *eg* mutation with respect to this phenotypic attribute. After the 20th generation of substitution backcrossing, the penetrance tests were repeated, yielding the following values of penetrance: GB-MIX 100%, GB-REP 99.5%, Wd-MIX 100%, Wd-REP 99%. The robustness of the estimates of selective effects must be considered in light of this slight departure from full penetrance. Many other *eg* mutants collected at approximately the same time showed consistently lower penetrance or penetrance that was more sensitive to the genetic background (A. G. CLARK and J. BUNDGAARD, unpublished results). Although induction of *eg* was demonstrated using MR chromosomes (GREEN 1982), we found no evidence for reversion of *eg* to wild type among 30,952 flies reared under dysgenic conditions.

Among flies that were *eg/eg* there was variation in the degree of wing spreading. Parent-offspring regression indicated a lack of genetic variation in these strains moderating the expression of *eg* [$b = 0.12$, not significant (NS)]. The effect of density on phenotypic expression was explored by scoring progeny of *eg/eg* flies obtained from the F_2 generation. The correlation of density with

phenotypic value was −0.076 (N = 165, NS). It can be concluded that the spread-wing phenotype is a reasonably good indicator of the *eg/eg* genotype, and errors incurred by the assumption have been quantified.

Segregation: Segregation tests performed at generations 0, 10 and 20 of the background substitutions were each replicated in two blocks of 18 vials performed 2 wk apart. In addition, flies from the original GB8S and Wd-4 lines were used in segregation tests performed after the 20th generation. Analysis of variance demonstrated that replicates were homogeneous in time. Chi square analysis of segregation data indicated no difference between male and female progeny ratios, and henceforth progeny data from segregation tests appear pooled over sexes.

Segregation tests are affected both by non-Mendelian segregation (meiotic drive) and by differences in egg to adult survivorship (viability or zygotic selection). As in any selection components analysis that does not sample zygotes, these two components cannot be fully resolved. For simplicity in discussion, results of segregation tests will be referred to as viability.

The influence of crowding on viability was examined by simple correlations. Progeny of single-pair crosses were reared in vials, and the correlation between density and viability within vials at generation 0 was −0.042 (N = 165, NS). Density independence of segregation is important for the independent estimation of fecundity and viability effects.

Now that a number of complicating factors have been ruled out, estimates of viability can be obtained from phenotypic counts of test crosses. In the case of crosses *eg/+* × *eg/eg* and *eg/eg* × *eg/+* the viability is defined as the ratio of the counts of *eg* to one plus the count of wild phenotypes (HALDANE 1956; ANXONALBEHERE, GOUX and PERIQUET 1982). This phenotypic ratio is multiplied by 3 in the case of *eg/+* × *eg/+* crosses, so that, in all cases, Mendelian segregation of adult phenotypes yields a value of 1 for the viability estimate. Figure 2 reports the segregation data from these three crosses at generations 0, 10 and 20 in the four genetic backgrounds. The total sample size for the segregation tests is 91,953 flies.

Analysis of variance was used first to compare the results from reciprocal crosses. Segregation from reciprocal crosses may differ due to sex-specific segregation distortion, an interaction with the sex chromosome or a cytoplasmic effect. Results of these tests are presented in Table 1. There were a few cases of significant reciprocal cross differences: at generation 0 and in the GB-MIX and Wd-REP backgrounds in generation 10. By generation 20 reciprocal crosses yielded the same segregation ratios, indicating that the earlier deviations were not inherently due to the *eg* locus. Since segregation distortion is generally limited to males in *Drosophila* (HARTL and HIRAIZUMI 1976), these results suggest that observed segregation ratios reflect viability differences.

The segregation tests at generation 0 reflect a clear background effect. The viability of *eg* homozygotes relative to heterozygotes determined from the cross *eg/+* × *eg/eg* was 0.770 ± 0.023, whereas the reciprocal cross yielded a viability estimate of 0.551 ± 0.021. In the first case, one round of recombination took place, reducing the association between *eg* and flanking loci, whereas, in the

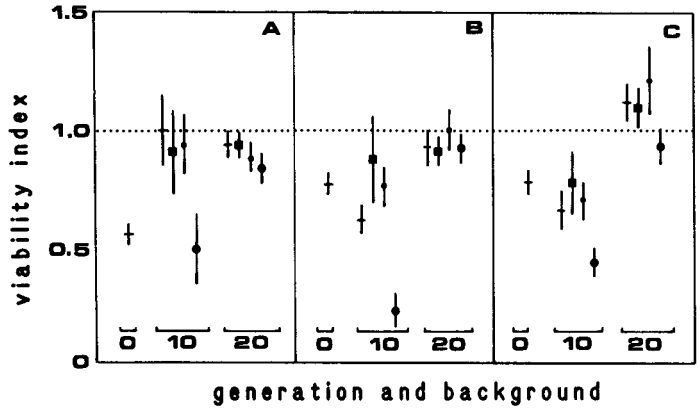

FIGURE 2.—Segregation results. The mean ± 2 SE of viability indices are plotted for generations 0, 10 and 20 in various backgrounds. At generations 10 and 20 the four different error bars represent from left to right: *GB-MIX*, *GB-REP*, *Wd-MIX* and *Wd-REP*. Box A represents results of crosses *eg/eg* females × *eg/+* males; Box B represents *eg/+* females × *eg/eg* males; Box C represents the *eg/+* × *eg/+* crosses.

TABLE 1

Estimates of viability of eg/eg *relative to* eg/+ *and standard errors*

Generation	Line	*eg/eg* × *eg/+*	*eg/+* × *eg/eg*	*eg/+* × *eg/+*
0		0.551 ± 0.021 (5778)	0.770 ± 0.023 (7636)	0.776 ± 0.026 (14,691)
10	GB-MIX	0.998 ± 0.076 (1120)	0.617 ± 0.031 (2077)	0.636 ± 0.041 (3,014)
	GB-REP	0.903 ± 0.090 (576)	0.876 ± 0.093 (1162)	0.774 ± 0.068 (1,668)
	Wd-MIX	0.932 ± 0.062 (1380)	0.756 ± 0.042 (2648)	0.693 ± 0.040 (2,994)
	Wd-REP	0.489 ± 0.076 (817)	0.218 ± 0.032 (2463)	0.433 ± 0.031 (1,758)
20	GB-MIX	0.938 ± 0.030 (2242)	0.926 ± 0.037 (1802)	1.121 ± 0.039 (2,902)
	GB-REP	0.938 ± 0.030 (3801)	0.908 ± 0.031 (3612)	1.094 ± 0.043 (5,960)
	Wd-MIX	0.882 ± 0.033 (3099)	0.997 ± 0.043 (3382)	1.212 ± 0.073 (4,419)
	Wd-REP	0.835 ± 0.030 (2846)	0.920 ± 0.029 (2580)	0.927 ± 0.037 (4,526)

A posteriori comparisons are indicated by lines: estimates not connected are different at $P < 0.05$. Sample sizes are given in parentheses.

latter case, no recombinants were formed. In the absence of recombination, homozygotes for *eg* were homozygous for the entire third chromosome (assuming that GB8S is isogenic). When *eg/+* male progeny from the second cross were mated with *eg/eg* sisters, the viability estimate was 0.514 ± 0.031, consistent with the absence of recombination. The reciprocal of the F_2 cross, however, allowed recombination between the GB8S and Wd-4 third chromosomes, and here the viability estimate was 0.711 ± 0.044.

The data from the crosses *eg/+* × *eg/eg* and *eg/eg* × *eg/+* represent estimates of the relative viability of the *eg* homozygotes to heterozygotes, assuming segregation is Mendelian. Progeny counts from *eg/+* × *eg/+* also reflect this viability difference, but results of the three crosses are expected to be consist-

TABLE 2

Parameters of sexual selection, fecundity and zygotic selection

	Codominant (hypothetical)				Dominant (observed)			
Sexual selection								
	♂				♂			
	eg/eg	*eg/+*	*+/+*		*eg/eg*	*eg/+*	*+/+*
♀ *eg/eg*	S_1	S_2	S_3	♀ *eg/eg*	$\tilde{S}_1$	$\tilde{S}_2$	$\tilde{S}_3$
♀ *eg/+*	S_4	S_5	S_6	♀ *eg/+*	$\tilde{S}_4$	$\tilde{S}_4$	$\tilde{S}_5$
♀ *+/+*	S_7	S_8	S_9	♀ *+/+*	$\tilde{S}_5$	$\tilde{S}_5$	$\tilde{S}_5$
Fecundity								
	♂				♂			
	eg/eg	*eg/+*	*+/+*		*eg/eg*	*eg/+*	*+/+*
♀ *eg/eg*	F_1	F_2	F_3	♀ *eg/eg*	$\tilde{F}_1$	$\tilde{F}_2$	$\tilde{F}_3$
♀ *eg/+*	F_4	F_5	F_6	♀ *eg/+*	$\tilde{F}_4$	$\tilde{F}_4$	$\tilde{F}_5$
♀ *+/+*	F_7	F_8	F_9	♀ *+/+*	$\tilde{F}_5$	$\tilde{F}_5$	$\tilde{F}_5$
Zygotic selection								
	eg/eg	*eg/+*	*+/+*		*eg/eg*	*eg/+*	*+/+*
	z_1	z_2	z_3		z_1	z_2	z_3

The distinct mating classes are (1) *eg* female with all *eg* progeny, (2) *eg* female with segregating progeny, (3) *eg* female with all wild progeny, (4) wild female with segregating progeny and (5) wild female with all wild progeny.

ent only if the *eg* allele is fully recessive in its viability effects. This was tested by a simple one-way analysis of variance, and results appear in Table 1.

Single-generation transition tests: To obtain meaningful estimates of the selection components, a model must be constructed that integrates the components into a transformation of genotypic frequencies through time. The model chosen here is a special case of a model presented by PROUT (1969, 1971a,b) and extended by CLARK and FELDMAN (1981). Table 2 depicts the model parameters. Let $N_j(j = 1\text{–}9)$ be the frequency of the jth mating type under the null hypothesis. In the case of Hardy-Weinberg equilibrium with random mating, if the frequency of the *eg* allele is $p = 1 - q$, then $N_j = p^4$, $2p^3q$, p^2q^2, $2p^3q$, $4p^2q^2$, $2pq^3$, p^2q^2, $2pq^3$ and q^4 for $j = 1$ to 9 respectively. Let $S_j(j = 1\text{–}9)$ be the sexual selection coefficients, describing the relative mating abilities, and $F_j(j = 1\text{–}9)$ represents the fecundities of the nine mating types. Both S_j and F_j are properties of mating pairs and allow interactions between the sexes. Let $Z_i(i = 1\text{–}3)$ be the zygotic selection coefficients for genotypes *eg/eg*, *eg/+* and *+/+*, respectively. These describe the relative probabilities of survival from zygote to reproductively mature adult. The transformation is:

$$g_i' = \frac{z_i \sum_j N_j S_j F_j K_{ij}}{\sum_i \sum_j z_i N_j S_j F_j K_{ij}}$$

where $g_i (i = 1\text{–}3)$ is the frequency of the ith genotype. K_{ij} is the Mendelian operator, defined as the fraction of progeny of mating type j that are genotype i. K_{ij} is in this case:

$$K = \begin{bmatrix} 1 & 0.5 & 0 & 0.5 & 0.25 & 0 & 0 & 0 & 0 \\ 0 & 0.5 & 0 & 0.5 & 0.5 & 0.5 & 1 & 0.5 & 0 \\ 0 & 0 & 0 & 0 & 0.25 & 0.5 & 0 & 0.5 & 1 \end{bmatrix}$$

This is slightly different from the formulation of PROUT (1969), because maternal and progeny phenotypes do not give sufficient information to distinguish all nine mating types and classes must be pooled. In particular, only five mating types can be distinguished, and the model parameters are reconfigured (Table 2). Now, we define $\tilde{N}_j$, $\tilde{S}_j$ and $\tilde{F}_j$ as before except j ranges from 1 to 5 and classes are pooled accordingly. Note that $\tilde{S}_4$, $\tilde{S}_5$, $\tilde{F}_4$ and $\tilde{F}_5$ will be the sexual selection and fecundity parameters weighted by the mating type frequencies. The Mendelian operator now becomes:

$$K = \begin{bmatrix} 1 & 0.5 & 0 & (p^3q + p^2q^2)/u & 0 \\ 0 & 0.5 & 1 & (p^3q + 2p^2q^2)/u & (2pq^3 + p^2q^2)/v \\ 0 & 0 & 0 & p^2q^2/u & (2pq^3 + q^4)/v \end{bmatrix}$$

where $u = 2p^3q + 4p^2q^2$ and $v = 4pq^3 + p^2q^2 + q^4$.

The critical question is whether it is possible to partition the components adequately, given that the observations $\tilde{S}_j$ and $\tilde{F}_j$ are incomplete realizations of S_j and F_j. Let S_{oj}, F_{oj} and Z_{oi} be defined as 1 for all i and j. These are the parametric values under the null hypothesis of no selection. Define:

$$g'_{iS} = \frac{z_{oi} \sum_j N_j S_j F_{oj} K_{ij}}{\sum_i \sum_j z_{oi} N_j S_j F_{oj} K_{ij}}$$

$$g'_{iF} = \frac{z_{oi} \sum_j N_j S_{oj} F_j K_{ij}}{\sum_i \sum_j z_{oi} N_j S_{oj} F_j K_{ij}}$$

$$g'_{iZ} = \frac{z_i \sum_j N_j S_{oj} F_{oj} K_{ij}}{\sum_i \sum_j z_i N_j S_{oj} F_{oj} K_{ij}}$$

It is then straightforward to calculate the changes in allelic frequency Δp_s, Δp_f and Δp_z attributable to each component from these genotypic frequencies:

$$\Delta p_s = (g'_{1s} + g'_{2s}/2) - (g_1 + g_2/2)$$

$$\Delta p_f = (g'_{1f} + g'_{2f}/2) - (g_1 + g_2/2)$$

$$\Delta p_z = (g'_{1z} + g'_{2z}/2) - (g_1 + g_2/2)$$

As BUNDGAARD and CHRISTIANSEN (1972) showed, the total change in allelic frequency in one generation is $\Delta p_t = \Delta p_s + \Delta p_f + \Delta p_z$, assuming segregation

is Mendelian and that these components are independent. In the cases in which S_j and F_j can be observed for $j = 1$–9, as in BUNDGAARD and CHRISTIANSEN (1972), estimates of Δp_s, Δp_f and Δp_z are direct. In the present case, the recessiveness of *eg* only allows observations of $\tilde{S}_j$ and $\tilde{F}_j$ (Table 3). Define $\tilde{g}'_{is}$, $\tilde{g}'_{if}$ and $\tilde{g}'_{iz}$ as before, replacing S_j and F_j by $\tilde{S}_j$ and $\tilde{F}_j$ and summing for $j = 1$–5. From these genotypic frequencies we can again estimate the allelic frequency changes $\Delta\tilde{p}_s$, $\Delta\tilde{p}_f$ and $\Delta\tilde{p}_z$. It is clear that $\Delta p_s \neq \Delta\tilde{p}_s$, $\Delta p_f \neq \Delta\tilde{p}_f$ and $\Delta p_z \neq \Delta\tilde{p}_z$ in general because information is lost when mating classes 4 and 5 are pooled. But the degree of departure between the two models is expected to be quite small unless differences among mating types within the pooled classes are very large.

To test the utility of $\Delta\tilde{p}_s$, $\Delta\tilde{p}_f$ and $\Delta\tilde{p}_z$ in estimating Δp_s, Δp_f and Δp_z a Monte Carlo method was used. For each of 100 populations, nine sexual selection parameters, nine fecundities and three viabilities were chosen at random from a uniform distribution over the interval [0.5, 1], allowing fitness differences of a factor of 2 in all components. Following the recurrence equations, estimates of the expected change in genotypic and allelic frequencies were obtained, and these yielded Δp_s, Δp_f and Δp_z. The mating type classes were then pooled as they would be in the *eg* experimental system, and $\Delta\tilde{p}_s$, $\Delta\tilde{p}_f$ and $\Delta\tilde{p}_z$ were calculated as described before. For each selection component as well as Δp_t correlations between the actual values and the estimates were calculated, and results appear in Table 4. The high correlations that appear even in this very conservative case appear to justify the use of this estimation procedure, and pooling mating classes did not appear to introduce a consistent bias.

Another issue concerns the statistical independence of the estimates of allelic frequency change. The Monte Carlo procedure generated independent values of Δp_s, Δp_f and Δp_z, whereas pooling mating types caused spurious correlations among $\Delta\tilde{p}_s$, $\Delta\tilde{p}_f$ and $\Delta\tilde{p}_z$. The correlation coefficient for $\Delta\tilde{p}_s$ *vs.* $\Delta\tilde{p}_f$ was 0.49 ($P < 0.01$), between $\Delta\tilde{p}_s$ and $\Delta\tilde{p}_z$ was 0.12 (NS) and between $\Delta\tilde{p}_f$ and $\Delta\tilde{p}_z$ was 0.20 ($P < 0.05$). These correlations underscore the need for caution in interpreting pleiotropic effects, except perhaps when estimates indicate changes in opposing directions.

Bootstrapping: Generally, confidence intervals are constructed for statistical estimates based on their theoretical distributions. Bootstrapping is a recently devised nonparametric method by which confidence intervals can be constructed without making assumptions about the theoretical distributions (EFRON 1979, 1981, 1982). In the present case, we wish to employ the empirical distributions of fecundities, mating choices and viabilities to place confidence intervals on the estimates of the changes in allelic frequency caused by each of the components. The single-generation transition experiments represent random samples from probability distributions of fecundities, mating choices and viabilities. Five different mating types can be distinguished in these experiments; therefore, let $C_j = S_j N_j C$ (where C is the total count of matings) be the counts of the different mating types and, hence, reflect sexual selection. The data do not represent an empirical distribution of C_j but rather represent a single sample from C_j. The implicit assumption in the analysis that follows is

TABLE 3

Summary of raw data from single-generation transition experiments

Generation	Background	Component	Mating class[a] 1	2	3	4	5
0	GB-8	S	3	23	3	43	40
		F	56.3 ± 18.1	88.2 ± 6.1	89.3 ± 3.7	79.8 ± 3.1	64.8 ± 3.4
		V		0.34 ± 0.02		0.23 ± 0.02	
	Wd-4	S	3	18	0	19	31
		F	54.3 ± 20.6	86.6 ± 7.5	0	80.4 ± 5.3	73.5 ± 4.4
		V		0.33 ± 0.02		0.17 ± 0.02	
10	GB-MIX	S	3	15	10	43	39
		F	76.7 ± 25.0	69.1 ± 7.8	82.0 ± 9.8	86.3 ± 4.8	82.4 ± 4.2
		V		0.36 ± 0.03		0.21 ± 0.02	
	GB-REP	S	1	6	8	37	38
		F	28.0 ± 0.0	56.5 ± 11.9	24.7 ± 10.3	49.6 ± 5.8	29.4 ± 5.2
		V		0.37 ± 0.05		0.16 ± 0.02	
	Wd-MIX	S	7	16	2	71	47
		F	12.3 ± 2.9	83.8 ± 10.9	90.5 ± 14.5	107.4 ± 3.9	103.3 ± 5.0
		V		0.41 ± 0.05		0.18 ± 0.02	
	Wd-REP	S	2	8	3	61	41
		F	14.0 ± 12.0	112.2 ± 8.4	62.0 ± 26.5	95.0 ± 5.0	82.9 ± 5.7
		V		0.23 ± 0.04		0.17 ± 0.01	
20	GB-MIX	S	8	18	3	52	32
		F	73.1 ± 5.9	77.9 ± 6.8	47.0 ± 20.5	75.2 ± 4.0	74.1 ± 5.7
		V		0.44 ± 0.03		0.29 ± 0.02	
		S	8	18	2	53	46
		F	50.1 ± 9.8	64.7 ± 6.1	27.0 ± 5.0	78.1 ± 3.9	56.1 ± 4.4
		V		0.39 ± 0.03		0.26 ± 0.01	
	GB-REP	S	10	26	4	61	42
		F	53.5 ± 9.8	57.1 ± 5.1	56.0 ± 19.6	65.4 ± 3.6	55.9 ± 3.8
		V		0.49 ± 0.03		0.26 ± 0.01	
		S	5	8	5	43	28
		F	29.0 ± 12.0	57.6 ± 11.1	53.6 ± 10.7	56.5 ± 3.9	46.9 ± 6.2
		V		0.47 ± 0.03		0.24 ± 0.01	
	Wd-MIX	S	2	18	4	49	35
		F	36.0 ± 16.0	63.2 ± 6.6	79.0 ± 5.7	59.2 ± 4.8	68.6 ± 6.1
		V		0.47 ± 0.02		0.31 ± 0.02	
		S	3	11	4	33	27
		F	50.0 ± 28.1	37.5 ± 7.2	11.7 ± 2.2	47.0 ± 5.0	55.1 ± 4.8
		V		0.51 ± 0.03		0.27 ± 0.02	
	Wd-REP	S	9	21	5	49	35
		F	27.7 ± 8.4	74.9 ± 7.4	87.8 ± 12.7	86.3 ± 4.4	82.3 ± 5.2
		V		0.41 ± 0.02		0.25 ± 0.02	
		S	1	6	5	48	49
		F	25.0 ± 0.0	56.8 ± 17.3	54.0 ± 7.2	62.5 ± 3.9	70.3 ± 4.3
		V		0.40 ± 0.05		0.23 ± 0.01	

[a]Mating classes are defined in Table 2. S represents the counts of the five mating classes, F is the mean ± standard error of fecundity and V is the mean ± standard error of the proportion of *eg* progeny. A total of 107,866 progeny flies are reported.

TABLE 4

Correlations between actual and estimated changes in phenotypic and allelic frequencies

	Sexual selection	Fecundity	Zygotic	Total
Phenotypic frequency	0.9486	0.8252	0.9662	0.9476
Allelic frequency	0.8950	0.7726	0.9316	0.9066

Correlations are based on Monte Carlo simulations of 100 populations. Correlations are not perfect because information is lost when mating classes are pooled.

that C_j is distributed multinomially. Let $F_j(j = 1\text{–}5)$ represent the five distributions of fecundity and $V_{ij} = K_{ij}Z_i$ be the distributions of the fractions of progeny of the jth mating type that are of genotype i (and hence represent viability). Let $R(C_j, F_j, V_{ij})$ represent the probability distribution of the change in allelic frequency after a one-generation transition. R is of course a function of the samples of F_j, C_j and V_{ij}, and an analytical formulation of the probability distribution of R is intractable. Bootstrapping allows an empirical determination of the distribution of R using the empirical distributions of C_j, F_j and V_{ij}. First, define the sample probability distributions $\hat{C}_j$, $\hat{F}_j$ and $\hat{V}_{ij}$ as those obtained by placing equal weight on each observation. Define C_{oj}, F_{oj} and V_{oij} as the respective distributions of the null case, *i.e.*, $F_{oj} = 1$ for all j implies that no fecundity differences were observed. To construct a bootstrap estimate of the fecundity effects, random samples were drawn with replacement from $\hat{F}_j$, with sample size equal to the observed sample size. For each bootstrap sample F_j^*, the change in allelic frequency $r^*(C_{oj}, F_j^*, V_{oij})$ was calculated. This process was repeated 1000 times, yielding a distribution of r^* denoted by $R^*(C_{oj}, F_j^*, V_{oij})$. The distribution $R^*(C_{oj}, F_j^*, V_{oij})$ approximates $R(C_{oj}, F_j, V_{oij})$ with accuracy depending on how closely $\hat{F}_j$ approximates F_j. Since no parametric assumptions were made in constructing $\hat{F}_j$, and it was derived from a sample of F_j, this approximation is likely to be quite good. The approximation improves with larger sample sizes, although the technique is not unduly sensitive to sample size (EFRON 1979). The confidence interval was placed on the estimate of allelic frequency change attributed to fecundity directly from the distribution $R^*(C_{oj}, F_j^*, V_{oij})$. The percentile method of constructing confidence intervals was used (EFRON 1982), because it entails no prior assumption of symmetric bootstrap distributions. These confidence intervals were found to be very stable with respect to the size of the bootstrap sample.

In a similar fashion, bootstrap estimates of allelic frequency change due to viability were determined. Bootstrap samples were drawn with replacement from $\hat{V}_{ij}$, and each sample was used to calculate a point $r^*(C_{oj}, F_{oj}, V_{ij}^*)$. The empirical distribution of r^* was constructed and $R^*(C_{oj}, F_{oj}, V_{ij}^*)$ was used to approximate $R(C_{oj}, F_{oj}, V_{ij})$. A confidence interval was constructed for the estimates of allelic frequency change due to viability directly from the bootstrap distribution.

Finally, bootstrap estimates of the effect of sexual selection must be made. Here, we have no empirical distribution $\hat{C}_j$ but instead assume that counts of

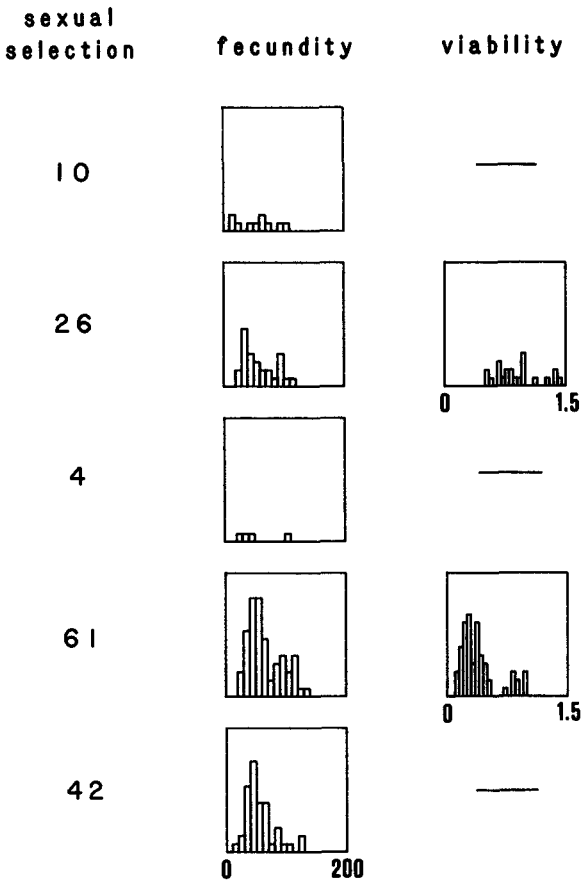

FIGURE 3.—Observed distributions of sexual selection, fecundity and viability in the first replicate of the generation 20 *GB-REP* single-generation transition experiment. The sexual selection column gives the observed counts of the five mating classes in this one experiment. The mating classes are, in order (1) *eg* female × *eg* male, (2) *eg* female × *eg*/+ male, (3) *eg* female × + male, (4) *eg*/+ female × either *eg*/*eg* or *eg*/+ male, (5) *eg*/+ female × + male or +/+ female × any male. Graphs of fecundity are to the same scale (0–200 progeny per female). No viability distributions are given for mating classes 1, 3 or 5 because progeny do not segregate in these crosses.

each of the five observed mating types follow a multinomial distribution. Multinomial samples were drawn from the observed sample of C_j, and each allowed a calculation of $r^*(C_j^*, F_{oj}, V_{oij})$. The empirical distribution of $r^*(C_j^*, F_{oj}, V_{oij})$ was $R^*(C_j^*, F_{oj}, V_{oij})$, and bootstrap estimates of the confidence interval of allelic frequency change due to sexual selection were obtained directly from this distribution. Figure 3 illustrates the empirical distributions of fecundity and viability from one single-generation transition experiment, and Figure 4 shows the resulting bootstrap distributions.

The theoretical justification of the bootstrapping procedure is presented by EFRON (1979, 1981, 1982). In the present case, bootstrapping was used to obtain estimates from distributions that are complex functions of several random variables. Although the method will induce small spurious correlations

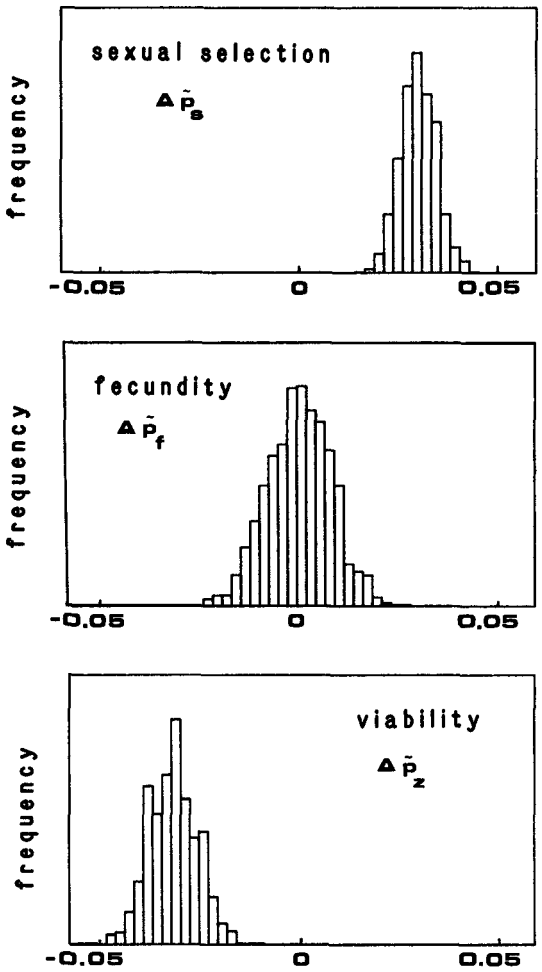

FIGURE 4.—Bootstrap distributions from the single-generation transition experiment represented in Figure 3. The distribution of $\Delta\tilde{p}_s$ is $R^*(C_j^*, F_{oj}, V_{oij})$, the distribution of $\Delta\tilde{p}_f$ is $R^*(C_{oj}, F_j^*, V_{oij})$ and the distribution of $\Delta\tilde{p}_z$ is $R^*(C_{oj}, F_{oj}, V_{ij}^*)$.

between selection components (as in the deterministic case outlined before), Monte Carlo methods verified that estimates were highly reliable. Bias was quantifiable (EFRON 1982) and can be defined in the case of $\Delta\tilde{p}_f$ as $E(R^*(C_{oj}, F_j^*, V_{oij})) - r(C_j, F_j, V_{ij})$, where the latter term is equivalent to the observed change in allelic frequency. These biases were consistently small (relative to the confidence intervals) and they varied in direction, so no attempt was made to correct them.

Results of the bootstrapping tests appear in Figure 5. Fecundity apparently had a negligible effect throughout the substitution backcrossing. The effects of sexual selection were consistently small in magnitude, with a tendency toward increasing *eg* allelic frequency in generations 10 and 20. The pattern of viabilities begins with strong selection reducing *eg* frequency and diminishing in strength as the experiment proceeds.

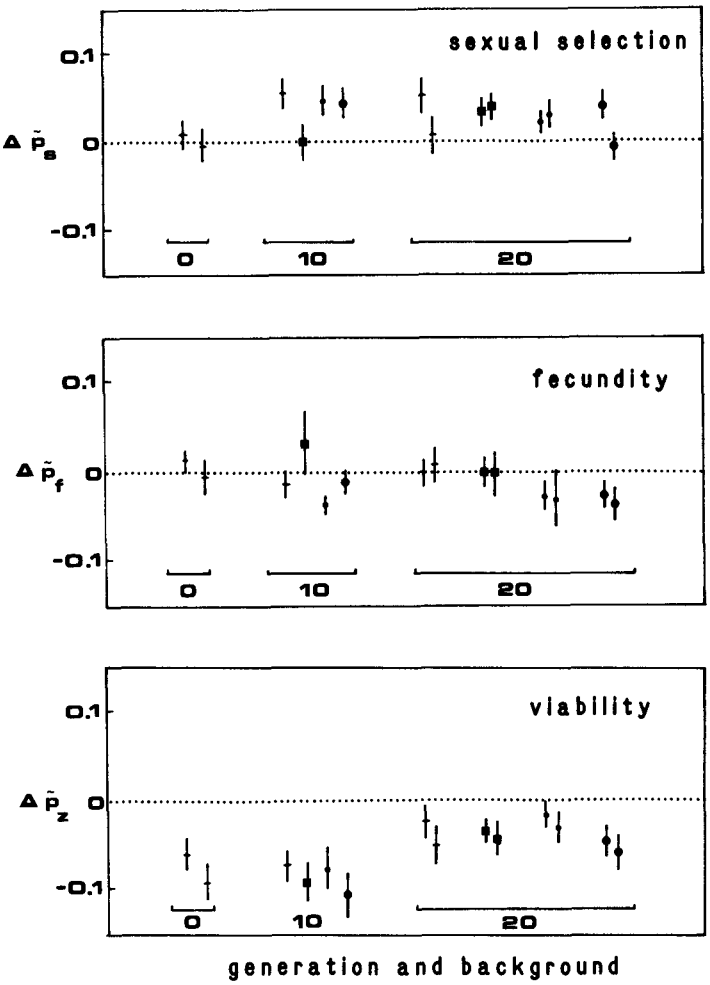

FIGURE 5.—Bootstrapping results. The means and 95% confidence intervals obtained directly from the bootstrapping distributions are plotted. At generation 0 the left bar is for the experiments in the *GB8S* cytoplasm and the right bar is for the *Wd-4* cytoplasm. At generation 10 the four bars are from left to right: *GB-MIX*, *GB-REP*, *Wd-MIX* and *Wd-REP*. The error bars for generation 20 are in the same order, and pairs of replicates are plotted beside each other.

Patterns of fitness: The analysis yields reliable estimates of allelic frequency change from one starting condition, but the ability to predict equilibrium behavior requires further knowledge. The decrease in *eg* frequency due to viability may for example be due to underdominance, overdominance or directional selection, and these three patterns differ greatly in equilibrium behavior. Due to the recessiveness of *eg* these patterns cannot be directly inferred, but indirect tests can be explored.

In the case of viabilities, data from different crosses can be used to estimate the viabilities of the two homozygotes relative to heterozygotes. Throughout this analysis it is assumed that segregation is Mendelian (a reasonable assumption when reciprocal crosses yield equal segregation ratios). Let the viabilities

of *eg/eg*, *eg/+* and *+/+* be designated v_1, $v_2 = 1$ and v_3, respectively. v_1 can be estimated directly from the segregations of progeny from the cross *eg/eg* × *eg/+* and its reciprocal. If p_1 is the fraction of *eg/eg* progeny from these crosses, then $\hat{v}_1 = p_1/(1 - p_1)$ is an obvious choice for the estimator, and its bias is very small for samples over 100 (HALDANE 1956). Assuming that the viability of *eg/eg* relative to *eg/+* is the same among progeny of the cross *eg/+* × *eg/+*, then if we let p_2 be the fraction of *+/+* progeny from the monohybrid cross we get the following phenotypic frequencies:

eg	+
$\dfrac{p_1}{p_1 + 2(1 - p_1) + p_2}$	$\dfrac{2(1 - p_1) + p_2}{p_1 + 2(1 - p_1) + p_2}$

If the counts of eg and + phenotypes are n_1 and n_2, then it is simple to construct the likelihood and solve for its maximum. This yields:

$$p_2 = [n_2 p_1 - n_1 2(1 - p_1)]/n_1$$

so the maximum likelihood estimate of v_3 is $\hat{v}_3 = p_2/(1 - p_1)$. The reliability of this estimator is questionable, because it is the ratio of scores from two different experiments, so a Monte Carlo study was done. One hundred pairs of viabilities v_1 and v_3 were generated at random by drawing from a uniform distribution on the interval [0.5, 1], and for each set binomial and trinomial sampling was performed to generate pseudodata representing progeny scores from crosses *eg/eg* × *eg/+* and *eg/+* × *eg/eg*, with $N = 500$ and $N = 2500$ progeny per cross. Estimates of $\hat{v}_1$ and $\hat{v}_3$ were calculated from the pseudodata, and resulting correlations between $\hat{v}_1$ and v_1 were 0.96 ($N = 500$), and 0.99 ($N = 2500$), whereas correlations between $\hat{v}_3$ and v_3 were 0.69 ($N = 500$) and 0.77 ($N = 2500$). The reliability of $\hat{v}_3$ was not sufficiently good to report detailed results here, despite the fact that it is a maximum likelihood estimator. The general pattern of viabilities was overdominant at generation 0 (with $\hat{v}_1 < \hat{v}_3$) and directional favoring the wild homozygotes at generations 10 and 20.

Bootstrapping indicated that fecundity differences had little effect on the polymorphism. To fully describe the pattern of fecundities, however, requires examination of the fecundity matrix and partitioning into effects due to each sex (CLARK and FELDMAN 1981). These data are not available due to the recessiveness of *eg*; however, results of the single-generation transition experiments can be used to compare fecundity distributions of *eg* and wild phenotype females. Scores from *eg* females represent three different mating types, whereas scores from wild females represent six different mating types. Analysis of variance was used, and results appear in Table 5. Of the ten comparisons, two were significant and both showed a fecundity disadvantage for *eg* females. The relatively small allelic frequency changes due to these fecundity differences suggest slight overdominance in the early stages of the substitution backcrossing.

Sexual selection can be inferred from mating type frequencies, but, again, in the present case a full description is not possible. Sexual selection among females can be thought of as a phenotype-dependent probability of mating and

TABLE 5

Comparisons of fecundities of eg *and wild phenotypes females from the single-generation transition experiments*

	eg females	+ females	*F*
Generation 0			
GB8S cytoplasm	72.59 ± 0.97	85.03 ± 1.80	5.43*
Wd-4 cytoplasm	76.12 ± 1.28	81.22 ± 1.50	0.85
Generation 10			
GB-MIX	74.54 ± 1.75	84.55 ± 1.07	2.36
GB-REP	37.67 ± 2.02	39.41 ± 0.96	0.03
Wd-MIX	64.32 ± 1.98	105.83 ± 0.99	27.01***
Wd-REP	85.54 ± 2.88	90.46 ± 1.02	0.18
Generation 20			
GB-MIX	73.41 ± 1.68	74.83 ± 1.01	0.05
GB-REP	56.08 ± 1.31	61.50 ± 0.84	1.12
Wd-MIX	63.62 ± 1.75	63.13 ± 0.98	0.04
Wd-REP	53.00 ± 2.40	66.43 ± 1.89	2.24

Reported are the mean ± SE scores of the number of progeny produced by individual females. * $P < 0.05$; *** $P < 0.001$.

producing progeny. Since matings are assessed by progeny, this procedure will confound sterility with mating propensity. Chi square analyses from 2 × 2 tables (*eg vs.* + phenotype females, and fertile *vs.* sterile) are presented in Table 6. Only two of the 14 chi square analyses are significant, and they represent a reverse in direction of sexual selection. The three different mating types involving *eg/eg* females allow an assessment of sexual selection operating in males. Results of chi square analyses determined from observed and expected mating type frequencies are presented in Table 6. The first significant chi square result represents overdominance, whereas the second indicates directional selection. The other two significant chi square results also represent a directional pattern of selection, consistent with the bootstrapping results (Figure 6). Sexual selection was also ascertained by performing chi square tests on the five observed mating type frequencies. Expected mating type frequencies were calculated based on the initial phenotypic frequencies, assuming random mating and no viability difference between *eg*/+ and +/+ flies (Table 6). The significant deviations seen here suggest that sexual selection is operating, but there may be a confounding viability effect.

Figure 6 presents results of the eight population bottles started after the 20th generation of backcrossing. Although the results of the single-generation transition experiments did not provide sufficient information to predict a stable polymorphism of *eg*, all eight populations appear to maintain a phenotypic frequency of *eg* of approximately 0.1. This result is consistent with the lack of differences among backgrounds in estimates of the Δp values obtained from the generation 20 transition experiments and the lack of differences among backgrounds in the segregation tests at generation 20.

Tests of gonadal dysgenesis revealed that the Wd-4 stock is clearly an *M* cytotype, whereas GB8S has a moderate *P* cytotype. Subsequent tests showed

TABLE 6

Chi square tests for direct ascertainment of sexual selection

	Females[a]	Males[b]	5 mating classes[c]
Generation 0			
GB8S cytoplasm	0.16	9.96	10.48*
Wd-4 cytoplasm	2.96	11.57*	22.13**
Generation 10			
GB-MIX	6.14*	2.56	10.59*
GB-REP	0.02	3.40	5.40
Wd-MIX	0.0	7.21*	18.76**
Wd-REP	1.52	4.37*	22.48**
Generation 20			
GB-MIX	0.60	0.58	9.72*
	5.96	1.27	5.06
GB-REP	1.92	1.18	7.72
	1.44	2.38	6.26
Wd-MIX	0.17	3.36	9.85*
	0.02	2.43	2.75
Wd-REP	2.66	1.17	4.95
	3.38	2.63	8.02*

[a] Female results are for *eg vs.* wild phenotype and fertile *vs.* sterile.
[b] Males results are from observed and expected counts of mating types with *eg/eg* females.
[c] Described in the text. All data are from the single-generation transition tests. The two replicates at generation 20 are reported separately.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

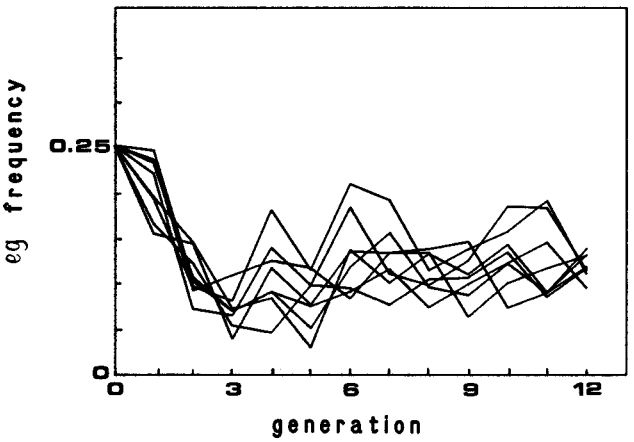

FIGURE 6.—*eg* phenotypic frequencies in discrete generation population bottles. Two replicates were started from each of the four genetic backgrounds after the 20th generation of backcrossing.

that there were stable in time. The *GB-MIX* and *Wd-MIX* lines had a definite *Q* cytotype by generation 20, whereas the *GB-REP* developed a *P* cytotype, and the *Wd-REP* developed an *M* cytotype. The replacement results are in agreement with ENGELS (1979), whereas the results of KIDWELL, NOVY and FEELEY (1981) suggest that mixed backgrounds usually develop a *P* cytotype.

DISCUSSION

Whenever Drosophila stocks of different origin are crossed in the laboratory, there is a risk of inducing a series of perturbing phenomena known as hybrid dysgenesis. Hybrid sterility and segregation distortion can be particularly troublesome when estimating components of fitness. In this study the crossing schemes Wd-REP and Wd-MIX generated dysgenic female hybrids (ascertained by gonadal dysgenesis), but there was no clear evidence that this had any affect on the relative fitnesses of the *eg* genotypes. The bootstrapping results also indicate that viabilities (ascertained by segregation) were similar over backgrounds. Segregation tests revealed a deficit of *eg* progeny in crosses with the Wd-REP background, yet this pattern is not consistent with hybrid dysgenesis because both reciprocal crosses yielded unusually low segregations. The pattern of low *eg* viability in the Wd-REP background persisted to generation 20 when there were no longer dysgenic females. Despite the certainty that hybrid dysgenesis was induced in these experiments, including possibly mutagenesis and transposition, the effects on the behavior of the *eg* polymorphism were negligible.

Insights into the genetic structure of fitness can be gained by first considering hypothetical results of these experiments. The lines GB8S and Wd-4 were initially highly homozygous due to inbreeding, and their hybrids would be expected to show the classic hybrid vigor. It is not unreasonable to expect that this marginal overdominance would be seen in fecundity, sexual selection and viability. If the *eg* mutation were neutral, then the GB-MIX and Wd-MIX crossing schemes would result in a progressive decline in linkage disequilibrium and, hence, a progressive decline in marginal overdominance. In the end the *eg* locus would be in linkage equilibrium with the genetic background, and despite the segregation of polygenic factors influencing fitness, the *eg* locus would appear neutral in all components.

The observed results differ markedly from this hypothetical case. There was no evidence for strong overdominance in fecundity, and the effect of fecundity variation on allelic frequency remained small throughout the experiment. Sexual selection was insignificant at generation 0 in the GB8S cytoplasm and thereafter showed a tendency toward increasing the frequency of *eg*. The bootstrapped viabilities were fairly consistent with the expectation, but there were still significant viability effects at generation 20. If *eg* is assumed to be neutral, this would represent fairly tight linkage between *eg* and the viability influencing loci. Segregation tests on the other hand seem to indicate the presence of factors in the genetic background with strong effects. Segregation ratios are particularly biased against *eg* in the Wd-REP background.

The estimation system used a number of approximations and assumptions, largely due to the recessiveness of *eg*. The GB8S and Wd-4 stocks were not isogenic, and the degree to which inbreeding leads to homozygosity depends on the genetic structure of viability determination. Likewise, the rate at which disequilibrium decays and the rates of which backgrounds are replaced in the substitution crossing schemes (Figure 1) depend on selective differences among alleles in the background. Although the bootstrapping technique allows esti-

mation of confidence intervals when parametric estimates do not exist, the assessment of selection components is somewhat limited in the present context. The ability to predict equilibrium behavior is lost by estimating allelic frequency changes rather than actual fitness parameters.

Despite these limitations, a number of aspects of the transmission of fitness were revealed. As outlined, the marginal fitnesses at the *eg* locus do not monotonically tend to neutrality but rather show large effects in different components at different times. The different times represent different configurations of the genetic background, and the effects may be due to few genes of major effect or interactions among polygenes. These effects seemed limited to single components. The strong segregation effect seen in the Wd-REP background in generation 10 is not reflected at all in fecundity for example. WRIGHT's (1977) call for "universal pleiotropy" has important theoretical implications, but we caution that, in the absence of a demonstrable biological mechanism, the inference of pleiotropic effects from selection components analyses requires knowledge of the genetic background.

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