THE UNIT OF SELECTION IN *DROSOPHILA MERCATORUM* I. THE INTERACTION OF SELECTION AND MEIOSIS IN PARTHENOGENETIC STRAINS[1]

ALAN R. TEMPLETON[2], CHARLES F. SING AND BARBARA BROKAW

The Society of Fellows and Department of Human Genetics, University of Michigan, Ann Arbor, Michigan 48104

Manuscript received January 27, 1975
Revised copy received July 14, 1975

ABSTRACT

An important problem in population genetics is the determination of the level of genetic organization to which fitness measures can be ascribed that yield an adequate description or prediction of the outcome of selection in populations. To study this problem, we used two strains of *Drosophila mercatorum* (S-1-Im and O-3-Im) that are capable of both sexual and parthenogenetic reproduction, a feature that allows us to experimentally control many factors which affect genetic variability. Both S and O reproduce parthenogenetically by "pronuclear duplication," a mechanism that retains normal meiosis (and hence crossing over and assortment) but results in homozygosity for all loci in a single generation. Since an isozyme survey indicated that S and O differ at a third of their loci, we hypothesized that S and O have adapted in genetically distinct fashions to the genetic environment of total homozygosity. This is tested by breeding females that are S-O hybrids for 100%, 60% and 40% of their genetic backgrounds, and scoring their respective parthenogenetic progenies for four isozyme and two visible markers. The data collected gave evidence for a coadaptation to total homozygosity involving non-additive and non-multiplicative interactions between non-alleles. As the perturbation of the parental coadapted genotypes by meiosis increases (i.e., the greater the degree of S-O hybridity), the level of genetic material which behaves as an additive/multiplicative fitness unit becomes larger. Selective neutrality of genetic variation may be an artifact of our failure to measure the proper genetic unit of selection.

AS used by Darwin, fitness is an attribute of an individual organism that measures its chances for reproductive success. However, in many populations individuals do not reproduce an exact copy of their multi-locus genotype, but rather pass on smaller levels of genetic organization to the next generation due to meiosis and gametic exchange. Consequently, although the individual is the "target of selection" (MAYR 1970), the individual's intact genotype may be a poor unit of measurement for the purpose of describing or predicting the genetic outcome of selection in a population.

[1] Supported by NSF Grant GB41278 ad AEC Contract AT(11-1)-1552.
[2] Present address: Department of Zoology, University of Texas, Austin, Texas 78712.

What then is the unit of selection? A glance at the literature of population genetics reveals that most evolutionary models either consider fitness to be a function of a single locus or to be an additive or multiplicative function of the fitnesses of the component loci forming a multi-locus genotype. Such models are often used for mathematical convenience, but can be given a theoretical justification. As CROW (1957) has pointed out, the two roles that a gene may have in an evolving population and in contributing to the fitness of an individual are not independent. Thus, in an organism like *E. coli*, a newly arisen mutant is "tested" by natural selection for its effects in a very limited number of genetic backgrounds, and we would expect an enhancement of epistatic interactions. For organisms such as Drosophila a mutant is tested against a large variety of genetic backgrounds resulting in selection for its "additive" effects. However, certain types of population structure, linkage, etc. can all limit the number of backgrounds against which an allele is tested even in a sexual population. The unit of fitness measurement which has utility in predicting the consequences of selection is undoubtedly some compromise between the forces which tend to break down and reorganize genotypes during reproduction and the cohesive forces of selection operating through individuals and of linkage, population subdivision, etc., which help retain genotype organization.

In this paper, the unit of selection is defined as the level of genetic organization (as identified by sets of marker loci) to which fitness measures can be applied that combine either additively or multiplicatively with the fitness measures of other such units in describing the outcome of selection in experimental populations. We chose to measure this unit in *Drosophila mercatorum*, a normally bisexual species capable of parthenogenetic reproduction in the laboratory (CARSON 1967a). The option of parthenogenetic reproduction in this species gives us precise control over the genetic background and other factors that may influence the unit of selection (SING and TEMPLETON 1975).

CARSON, WEI and NIEDERKORN (1969) demonstrated that the parthenogenetic strains of *D. mercatorum* are diploid and automictic; i.e., they retain normal meiosis. Diploidy is restored either by fusion of two haploid egg pronuclei or by duplication of a single pronucleus followed by fusion (Pronuclear Duplication, Figure 1). With fusion, heterozygosity can be maintained through the normal operation of meiosis (NACE, RICHARDS and ASHER 1970), but pronuclear duplication results in total homozygosity in a single generation. Different strains have characteristic proportions of pronuclear duplication that vary from 78% to 99% of the eggs (CARSON 1973). Parthenogenetic females can also reproduce sexually if males are made available.

We decided to exploit the potential of *D. mercatorum* by first conducting experiments on stocks that reproduce almost exclusively by pronuclear duplication. The purpose of this work is to study selection at a multi-locus level in parthenogenetic populations in which genetic diversity is created by meiosis alone, and to describe the unit of selection if selection is present. Furthermore, the extent of the genetic diversity created by meiosis will be experimentally manipulated so that any interaction between selection and the degree of meiotic perturbation upon the genetic unit of selection may be determined.

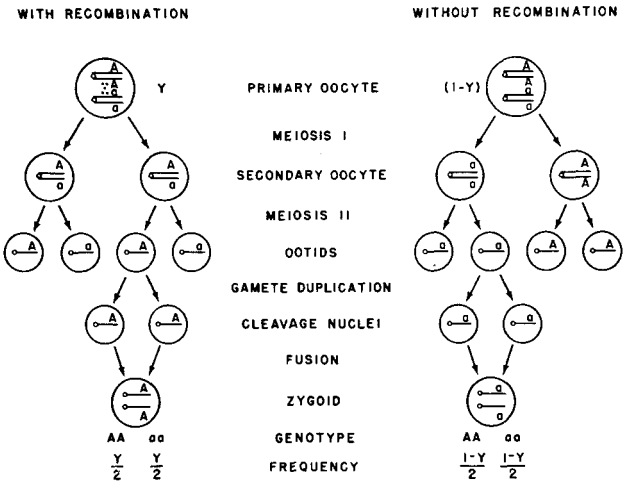


FIGURE 1.—Diagrammatic representation of the meiotic events of pronuclear duplication.

MATERIALS AND METHODS

Stocks and markers

One bisexual and two parthenogenetic stocks of *D. mercatorum* were obtained from DR. HAMPTON L. CARSON. The bisexual stock is designated "*v pm vl*" and has visible recessive markers on all three of the major autosomes. (*D. mercatorum* has the sex chromosome, a metacentric autosome, 2 acrocentric autosomes and a dot.) Two of these markers—*v*ermillion and *pl*um—are eye color mutants and the third is characterized by *v*ein*l*ess wings (CARSON and SNYDER 1973). This stock is also polymorphic for a sex-linked recessive called *spot*less that causes the absence of a spot that normally surrounds the base of the posterior reclinate orbital bristle. With *sl*, all the major chromosomes of the "*v pm vl*" stocks are visibly marked.

The two parthenogenetic stocks are designated S-1-Im and O-3-Im (CARSON 1967a). S-1-Im was derived from flies caught in El Salvador and has been reproducing parthenogenetically since 1961. O-3-Im comes from Oahu and was established in 1965. CARSON (1973) estimated the percent of eggs restoring diploidy *via* pronuclear duplication in O-3-Im as 94% with the remainder undergoing fusion. TEMPLETON and ROTHMAN (1973) estimated the lower bound for the percent pronuclear duplication as 94% in Iso-8-S-1-Im (obtained from S-1-Im through 8 single female generations) and the upper bound as 96%. O-3-Im was also put through 8 single female generations to obtain Iso-8-O-3-Im. With these levels of gamete duplication, eight single female generations essentially ensures that only a single haploid genome-type exists within each strain. An isozyme survey of 15 loci (*Est-A, Est-B, Acph, Tet, Ox., XDH, SDH, ICDH, α-GPD, MDH, GA-3-PD, ADH, PGM, G-6-PD, 6-PGD, PHI*) using the starch gel systems described in BREWER (1970) revealed allelic differences between the resulting isogenic stocks at five loci (*Est-A, Est-B, XDH, Acph, G-6-PD*), indicating the stocks differ at about a third of their loci. Furthermore, Iso-8-S-1-Im is homozygous *sl/sl* and Iso-8-O-3-Im is homozygous *sl+/sl+*. Esterases A and B, Xanthine dehydrogenase and Glucose 6 phosphate dehydrogenase (hereafter referred to as *A, B, X* and *G*) were used in these experiments as genetic markers since they could be run on the same gel and bridge buffer systems. Linkage studies indicated that *G* is sex-linked while *A, B* and *X* are all linked to *v* on the metacentric autosome (Figure 2).

Additional stocks necessary for this study were bred from these three basic stocks. The first of these is the "bridge" stock S-*sl v pm vl*-Br_i, bred with the crosses:

S-sl v pm vl-Br_i ♂ × Iso-8-S-1-Im ♀ (which is *sl/sl*)

↘

S-sl v pm vl-Br_i ♂ × F_1 ♀

↙

S-*sl v pm vl* Br_{i+1}♂ × Iso-8-S-1-Im ♀ etc., i=0, . . . , 11

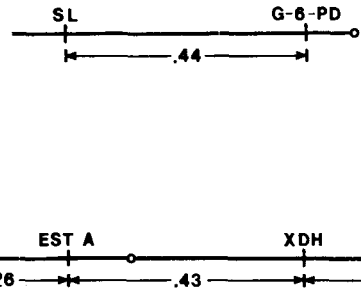


FIGURE 2.—The chromosome map for two of the four major chromosomes of *Drosophila mercatorum*.

Where S-*sl v pm vl*-Br_0 refers to the *v pm vl* stock. Every time the cycle goes through an F_1 female, crossing over will put the visible markers on a background that becames more and more S-l type. After the 12th cycle, a single Br_{12} female was forced to reproduce parthenogenetically. Her impaternate offspring were then backcrossed to a single S-*sl v pm vl*-Br_{12} male, a sib of the female parent, to form the S-*sl v pm vl*-Br_{12} sexual stock used in the present study (established July, 1972). Several generations after its establishment, 29 S-*sl v pm vl*-Br_{12} females isolated as virgins produced an average of 11.04 viable offspring per female within 10 days after eclosion as compared to 14.54 for 24 Iso-8-S-1-Im females. Hence, S-*sl v pm vl*-Br_{12} flies have a genome well adapted to parthenogenetic reproduction and its genetic consequences. A single virgin female from this stock was used to found the parthenogenetic stock S-*sl v pm vl-Im* in December, 1972. All of these stocks as well as Iso-8-S-1-Im will hereafter be referred to as "S" stocks, while "O" will refer to Iso-8-O-3-Im and related stocks.

The *v pm vl* males were also bridged to Iso-8-O--3-Im females. At the third bridge cycle, matings were made between O-*sl v pm vl* Br_3 males and females. The progeny of one such pair mating proved to be homozygous for the four isozyme markers used in the experiment: *B* and *G* for the S-type allele, *A* and *X* for the O-type allele. This pair mating was the founder for a marker stock designated M-*sl v pm vl* (established in December, 1972).

The *v pm vl* stock was used to generate an additional bridge stock, O-Br_i, as follows:

v pm vl ♂ × Iso-8-O-3-Im ♀

O-Br_1 ♂ ↙ × Iso-8-O-3-Im ♀

O-Br_2 ♂ ↙ × *v pm vl* ♀ (several pair matings).

An O-Br_2 male was selected from the pair matings that yielded only wild-type offspring. Since there is no crossing over in the male, this selected O-Br_2 male had to have its *X* chromosome and all three pairs of the major autosomes of the O-type. One of the dots has to be of the O type and the other dot could either be O or the original *v pm vl* type with equal probability. To ensure both dots are of the O type, further backcrosses were made:

O-Br_i ♂ × Iso-8-O-3-Im ♀

O-Br_{i+1} ♂ ↙ × Iso-8-O-3-Im ♀ etc.

The probability that both dots are of the O type is $1-(1/2)^{i-1} > .99$ for $i \geq 8$. This scheme produces males that are genetically identical to O females except for the *Y* chromosome and, with a very small probability, one of the dots.

Experimental design

Since the O and S stocks evolved under totally homozygous conditions, we hypothesize they have genome types adapted to this genetic environment. This hypothesis is tested by perturbing their genotypes away from their original state and then observing with several marker loci the

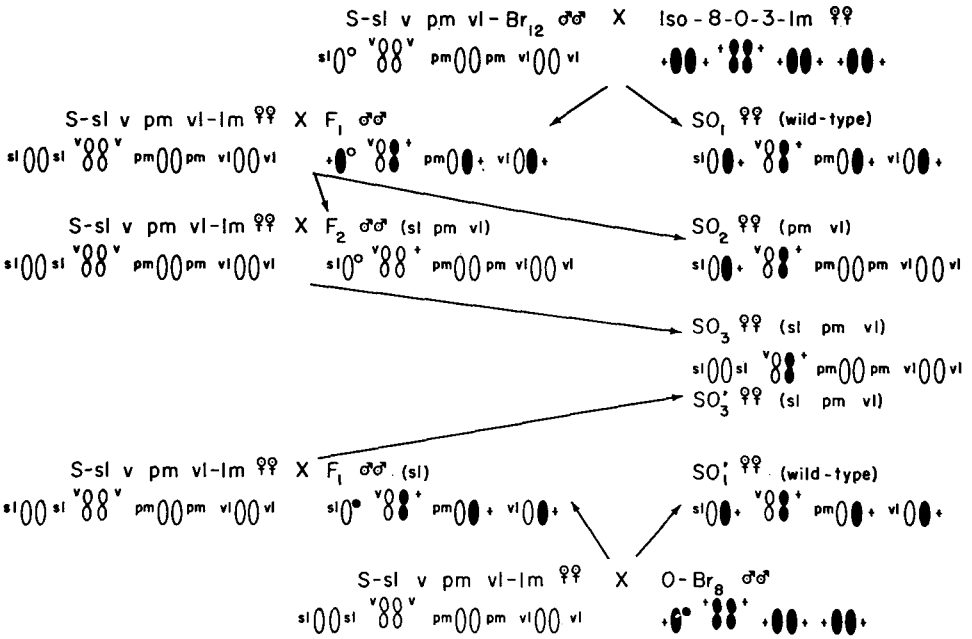


FIGURE 3.—Mating scheme used to generate the experimental treatments and a diagrammatic representation of the chromosomal constitution of the three types of SO hybrid females. There are five major chromosome arms of approximately equal size with the metacentric "*v*"-marked autosome having two arms and the others only one. A small pair of dot autosomes is not shown in this diagram.

fitness effects of such a perturbation in a totally homozygous and in a heterozygous genetic environment. The O and S genotypes are perturbed by breeding females that are hybrid SO for selected portions of their genomes, as shown in Figure 3, and then allowing meiosis to proceed. We created three types of SO hybrids: SO_1 females which are hybrid for 100% of their genotype, SO_2—60% and SO_3—40%. The 60% and 40% hybrids were homozygous for the S genotype in the non-hybrid portion of their genotype. As meiosis proceeds in the öogonia of these SO females, the original O and S genomes are changed in their allele composition by both assortment of chromosomes in the 100% and 60% treatments and recombination between homologous chromosomes in all perturbation levels. Due to the stochastic nature of meiosis, some of these recombinant genomes will be very similar in allele content to a homozygous parental genotype while others will be very far removed from the original O and S genotypes.

To restore diploidy after meiosis, some of the SO females from each perturbation level were placed in shell vials within 24 hours after eclosion without males to reproduce parthenogenetically. For the 100% perturbation fifteen virgin SO_1 females were placed in a shell vial. The number of viable parthenogenetic F_2's produces by SO_1 females was low, so we decided to let them reproduce 17 days—the generation span—before discarding them. The SO_1 females were transferred to a fresh vial after 7 days, and to a third vial after another five days. After five days in the third vial, the SO_1 females were removed and frozen for starch gel electrophoresis in order to ensure they were indeed SO hybrids. This 7-5-5 day pattern was chosen to equalize the number of progeny in each vial and prevent larval overcrowding. There was very little mortality among the SO_1 females during the 17 days, with usually none dying and at most three. The SO_2 and SO_3 females were capable of producing more viable parthenogenetic offspring than the SO_1, so the number of females per vial was often reduced from 15 to between 8 to 12 to

TABLE 1

Experimental treatments with the number of progeny produced parthenogenetically by S-O hybrid females at the three perturbation levels

Perturbation level	Type of female parents	No. of vials	Total no. of parth. progeny	No. of parth. progeny homo. for all isozyme loci	Percent progeny heterozygous at 1 or more loci
100%	SO_1	12	344	317	7.85%
60%	SO_2	10	870	844	2.99%
40%	SO_3	11	952	925	2.84%
40%	SO_3'	11	1472	1412	4.08%
Total		44	3638	3498	3.85%

avoid larval overcrowding. The number of replicates for each treatment level and the number of progeny produced are given in Table 1.

Shortly after the females were discarded from the third vial, the parthenogenetic progeny began to emerge. These were collected every day to minimize adult mortality. The collected progeny were anaesthetized with ice and scored for the visible markers *v* and *sl*. The progeny were then frozen and scored at a later date for the isozyme markers, *A*, *B*, *X* and *G*.

To ensure that the SO hybrids restore diploidy via duplication, several parthenogenetic progeny of SO_1 females were isolated and individually backcrossed to *sl v pm vl* males to check for heterozygosity at these four unlinked visible marker loci. A total of 236 flies were scored and 229 were homozygous for all four loci. Using the technique given by TEMPLETON and ROTHMAN (1973), the lower bound for the frequency of duplication is estimated to be 0.95 and the upper bound 0.96. Consequently, the vast majority of the parthenogenetic progeny of SO_1 females will be totally homozygous. Furthermore, progeny heterozygous for one or more of the four isozyme loci are excluded from further analysis. In a later paper we will show that most of the non-duplication eggs restore diploidy by central fusion and the *A* marker seems to be closely linked to the centromere. As shown by CARSON (1967b), central fusion will almost always result in heterozygosity for loci closely linked to the centromere, and in fact nearly all flies excluded from the analysis were heterozygous for *A*. The average percentage of flies excluded is 3.85% (Table 1). Since the percentage of duplication in S and SO_1 is 96% (under the assumption of central fusion in the remaining eggs) we are confident that most parthenogenetic progeny homozygous for all isozyme markers are indeed the result of pronuclear duplication, and the selective forces associated with total homozygosity (if any) should be operative. Any interactions between selection and degree of meiotic perturbation may be studied by contrasting the results from the 100%, 60% and 40% perturbation levels.

Other SO females from each perturbation level were mated to various genetically marked males such that high levels of heterozygosity were created in their sexual progeny. The number of progeny produced sexually was always greater than that produced parthenogenetically, so only 3 SO females were used in the control vials to avoid larval overcrowding. The 7-5-5 pattern was retained. Four males homozygous for the 6 scored markers were mated to these females. These crosses are given in Table 2. As can be seen, different types of marker males were used, but in all cases the isozyme results indicated the resulting sexual progeny should be heterozygous for at least 15% of their loci. Under these conditions, the assumption is made that the selective forces associated with the totally homozygous genetic state are eliminated or, at least, markedly reduced. In any case, contrasts between genotype frequencies of the parthenogenetic progeny with frequencies of the maternally derived genotype component of the sexual progeny should yield evidence for selection that is associated with the nature of the genetic environment. Furthermore, the sexual progeny were used as controls to check for segregation distortion and to estimate recombination frequencies.

TABLE 2

Bisexual control crosses of S-O hybrid females with genetically marked males

Perturbation level of female parent	Cross designation	Cross	Genotype of ♂ parent *B*	*A*	*X*	*v*	*G*	*sl*	No. of replicates	Progeny separated by sex	No. of progeny
100%	C_1	$3SO_1$ ♀ ♀ × 4 S-*slv pm vl* Br_{12} ♂ ♂	*F*	*S*	*F*	*v*	*S*	*sl*	3	no	343
	C_2	$3SO_1$ ♀ ♀ × 4 S-*slv pm vl* Br_{10} ♂ ♂	*F*	*S*	*F*	*v*	*S*	*sl*	14	no	923
100%	C_3	$3SO_1$ ♀ ♀ × 4 *v pm vl* ♂ ♂	*F*	*S*	*F*	*v*	*S*	*sl*	4	no	314
	C_4	$3SO_1$ ♀ ♀ × 4 S-*slv pm vl* Br_{10} ♂ ♂	*F*	*S*	*F*	*v*	*S*	*sl*	13	yes	1155
	C_5	$3SO_1'$ ♀ ♀ × 4 M-*slv pm vl* ♂ ♂	*F*	*F*	*S*	*v*	*S*	*sl*	3	yes	977
60%	C_6	$3SO_2$ ♀ ♀ × 4 S-*slv pm vl* Br_{10} ♂ ♂	*F*	*S*	*F*	*v*	*S*	*sl*	4	yes	704
	C_7	$3SO_2$ ♀ ♀ × 4 M-*slv pm vl* ♂ ♂	*F*	*F*	*S*	*v*	*S*	*sl*	2	yes	674
40%	C_8	$3SO_3$ ♀ ♀ × 4 M-*slv pm vl* ♂ ♂	*F*	*F*	*S*	*v*	*S*	*sl*	7	yes	1746
	C_9	$3SO_3'$ ♀ ♀ × 4 M-*slv pm vl* ♂ ♂	*F*	*F*	*S*	*v*	*S*	*sl*	2	yes	887
									Total		7723

STATISTICAL MODELS AND METHODS OF ANALYSIS

A variety of statistical tools will be used to characterize the sexual controls, to compare the controls with the parthenogenetic progeny, and to test hypotheses. Some of these tests are standard ones and will be referred to as they are used. However, the procedures for estimating fitnesses of the parthenogenetic progeny will be reviewed here since this will clarify what is operationally implied by the "unit of selection".

Data were collected on six loci per fly, although for a few flies one or more isozyme systems sometimes proved unscorable. It is therefore possible to estimate the six-locus genotypic fitnesses. There are 64 six-locus, homozygous genotypes, but some of our treatment levels had only 201 flies scored for all six loci. It is obvious that the data simply do not justify analyzing selection at this level. A compromise is necessary between the number of genotypes and the sample size that allows meaningful statistical analysis. We chose to consider only the analysis of one-, two- and three-locus genotypes.

Under pronuclear duplication, a hybrid female heterozygous for a particular locus should produce equal numbers of 0- and S-type homozygous progeny in the absence of selection. However, suppose there is some selection between restoration of diploidy and scoring. The 0 homozygote (AA) could then be given a relative fitness of 1 while the S homozygous (aa) could be given a fitness of w, some constant not necessarily equal to one. Such a model assumes that all selection is a function of the genotype only. This is justified for three reasons. First, there are no genetically determined fecundity differences in the female parents because the isogenicity of the O and S stocks ensures that all females used for a particular experimental treatment are genetically identical. Secondly, selection may only operate between the time of meiosis and sampling. If any selection is operating on meiosis *per se* (e.g., meiotic drive) it will show up in the controls also since they have female parents of the same genotype. Consequently, in the absence of contrary evidence from the controls, all selection may be regarded as occurring

between the formation of the haploid pronuclei (which in a duplication system is equivalent to the final determination of the individual's genotype) and the time of sampling—that is, the viability of a particular totally homozygous genotype. Thirdly, the progeny were raised in temperature- and light-controlled incubators under low densities in order to standardize environmental influences.

Suppose n adults are scored for the locus under consideration, and x_1 are homozygous for A and x_2 for a. The probability distribution of x_1,x_2 is

$$\binom{n}{x_1}\,[1/(2\bar{w})]^{x_1}[w/(2\bar{w})]^{x_2} \tag{1}$$

where $\bar{w} = (1 + w)/2$. The $\ln(\log_e)$ likelihood is proportional to

$$x_2\ln(w) - n \cdot \ln(\bar{w}) \tag{2}$$

and the maximum likelihood estimate of w is $\hat{w} = x_2/x_1$. A standard ln-likelihood ratio test can be used to test the hypothesis of selection ($w \neq 1$) *versus* the null hypothesis of no selection ($w = 1$);

$$2[L(\hat{w}) - L_0] \rightarrow \chi_1^2 \tag{3}$$

where $L(\hat{w})$ is equation (2) evaluated at $\hat{w}$, L_0 is (2) exaluated at $w = 1$, $\rightarrow$ denotes convergence in law, and χ_1^2 denotes a variable distributed as a chi-square with 1 degree of freedom. There is no additional test for the goodness of fit of the selection model since there is only one independent observation and one parameter—hence, the estimated $\hat{w}$ will fit the data exactly.

For the two-locus systems, the statistical model becomes more complex, as does the nature of the inference one can make. Consider two loci with recombination frequency r. Let capital letters designate the 0-type alleles and small letters the S. An SO hybrid female will produce four types of gametes: AB and ab each with frequency $p_1 = p_2 = (1 - r)/2$, and Ab and aB each with frequency $p_3 = p_4 = r/2$. Suppose these gametes are duplicated to form diploids. The probabilities of the various diploids surviving to adulthood are once again assumed to be constants that depend only on the genotype. The following fitness model may be used:

Genotype	AB	ab	Ab	aB
Relative fitness	$w_1 = 1$	w_2	w_3	w_4
Numbers in sample of n	x_1	x_2	x_3	x_4

The two-locus ln-likelihood corresponding to (2) is proportional to

$$\sum_{i=1}^{4} x_i\ln(w_i) - n \cdot \ln(\bar{w}) \tag{4}$$

where $\bar{w} = \sum_{i=1}^{4} p_i w_i$. Given the p's, the maximum likelihood estimates of the w's are:

$$\bar{w}_i = (x_i p_1)/(x_1 p_i).$$

This estimated fitness model will fit the observed data exactly. The null hypothesis of no selection may be tested with

$$2[L(\hat{w}) - L_0] \rightarrow \chi_3^2 \tag{5}$$

where $L(\hat{w})$ is (4) evaluated at the $\hat{w}$'s and L_0 is (4) at $w_i = 1$ for all i.

To gain further insight into the meaning of these models, consider the linear fitness model which describes the two-locus homozygous genotypes:

		Alleles at second locus	
		B	b
Alleles at	A	$\mu+\alpha+\beta+\varepsilon$	$\mu+\alpha-\beta-\varepsilon$
first locus	a	$\mu-\alpha+\beta-\varepsilon$	$\mu-\alpha-\beta+\varepsilon$

Thus, the four fitnesses of the unrestricted model are reparameterized in terms of a grand mean, μ, an additive effect for the A allele, α, ($-\alpha$ is the additive effect for the a allele), an additive effect for the B allele, β, and an interaction term, ε. However, only three of the four observations are independent. The number of independent parameters is reduced to three by setting $\mu + \alpha + \beta + \varepsilon = w_1 = 1$.

To test the significance of the epistatic term ε, the constraint $\varepsilon = 0$ is introduced into the estimation procedure to yield:

$$\hat{w}'_i = x_i(x_1 + x_2)/[x_1(x_i + x_j)] \qquad j = \begin{cases} i+1 \text{ if } i \text{ odd} \\ i-1 \text{ if } i \text{ even.} \end{cases}$$

The constraint gives two independent fitnesses, so the test of the null hypothesis versus the fitness model with no epistasis is

$$2[L(\hat{w}') - L_0] \to \chi^2_2 \tag{6}$$

Furthermore, the test of the goodness of fit to the data of the non-epistatic model is

$$2[L(\hat{w}) - L(\hat{w}')] \to \chi^2_1 \ . \tag{7}$$

The only difference between the unrestricted fitness model and the nonepistasis model is the constraint $\varepsilon = 0$. Hence, test (7) may be regarded as a test of the hypothesis $\varepsilon = 0$ *versus* $\varepsilon \neq 0$. Alternatively, (7) tests the goodness of fit of an additive fitness model in which all fitnesses may be adequately predicted from the marginal additive components α and β. If test (7) indicates that the additive model gives an adequate fit to the data, we conclude that *the additive units of selection are the genetic regions marked by individual loci.*

An alternate fitness model is:

		Alleles at second locus		
		B	b	Marginal
Alleles at	A	1	m_2	1
first locus	a	m_1	$m_1 m_2$	m_1
	Marginal	1	m_2	

where the fitness of the two-locus genotypes is determined by multiplication of the marginal fitnesses. The maximum likelihood estimates of m_1 and m_2 are found using a likelihood scoring procedure. The ln-likelihood can then be evaluated under this model and the following tests made:

$$2[L(\hat{m}) - L_0] \to \chi^2_2 \tag{8}$$

$$2[L(\hat{w}) - L(\hat{m})] \to \chi^2_1 \ . \tag{9}$$

Statistic (8) tests whether or not there is significant multiplicative selection and (9) tests the goodness of fit of the multiplicative model. If (9) indicates a good fit, we conclude that *the multiplicative units of selection are the genetic regions marked by the individual loci.*

The two-locus model is readily extended to the case of three loci. Five three-locus fitness models are used in this paper, and their parameters are estimated and tested using maximum likelihood techniques analogous to the two-locus case. These models are given in Table 3 where the markers of all three-locus systems are grouped such that the first two are always on the same chromosome arm and the third is on a different arm or unlinked; or the first two are on the same chromosome but different arms and the third is unlinked. Note that fitness models III and IV have no restrictions between the first two markers, but constrain the third marker to interact either in an additive (III) or multiplicative (IV) fashion with the *unit* defined by the first two markers.

Until now we have assumed that the p's are known. For the eight unlinked two-locus systems we may *a priori* set $p_i = 0.25, i = 1, \ldots, 4$. For the remaining seven two-locus systems, the p's depend upon the crossover frequency, which may be estimated from the controls as $r =$ number of recombinants $/n$ (sample size). The estimated variance of r is $s_r^2 = r(1-r)/n$. Then $\hat{p}_1 = \hat{p}_2 = (1-r)/2$ and $\hat{p}_3 = \hat{p}_4 = r/2$. Note there is only one independent $\hat{p}$. Thus, to get an estimate of the sampling error involved in this estimate, we need only consider the variance of $\hat{p}_1$ which is $s_p^2 = s_r^2/4$. For the sample sizes considered, s_r^2 is going to be very small and s_p^2 even smaller. Thus, we may substitute the $\hat{p}$'s into the likelihood without introducing any significant sampling error. However, if our assumption of neutrality in the controls is false, the fitness estimators will be biased. Similar considerations hold for the three-locus systems involving two linked and one unlinked locus since their p's are a function of only one recombination frequency. For the four systems which involve three linked loci, three recombination parameters estimated from the controls are required to evaluate the p's. In general, these four systems should be the most sensitive to sampling error; but with large sample sizes this will not be a problem.

TABLE 3

Fitness models used for the three-locus genotypes

Genotype (First two loci are always on same chromosome)	Unrestricted	Fitness under model: I. Gene additive	II. Gene multiplicative	III. Chromosome additive	IV. Chromosome multiplicative
ABC	1	$\mu+\alpha+\beta+\gamma=1$	1	$\mu+\alpha+\beta+\varepsilon_{12}+\gamma=1$	$M_1=1$
abc	W_2	$\mu-\alpha-\beta-\gamma$	$M_1 \cdot M_2 \cdot M_3$	$\mu-\alpha-\beta+\varepsilon_{12}-\gamma$	$M_2 \cdot m$
Abc	W_3	$\mu+\alpha-\beta-\gamma$	$M_2 \cdot M_3$	$\mu+\alpha-\beta-\varepsilon_{12}-\gamma$	$M_3 \cdot m$
aBC	W_4	$\mu-\alpha+\beta+\gamma$	M_1	$\mu-\alpha+\beta-\varepsilon_{12}+\gamma$	M_4
ABc	W_5	$\mu+\alpha+\beta-\gamma$	M_3	$\mu+\alpha+\beta+\varepsilon_{12}-\gamma$	m
abC	W_6	$\mu-\alpha-\beta+\gamma$	$M_1 \cdot M_2$	$\mu-\alpha-\beta+\varepsilon_{12}+\gamma$	M_2
AbC	W_7	$\mu+\alpha-\beta+\gamma$	M_2	$\mu+\alpha-\beta-\varepsilon_{12}+\gamma$	M_3
aBc	W_8	$\mu-\alpha+\beta-\gamma$	$M_1 \cdot M_3$	$\mu-\alpha+\beta-\varepsilon_{12}-\gamma$	$M_4 \cdot m$

RESULTS

Controls

In the absence of selection the progeny of the sexual crosses provide "null" frequencies for the genotypic classes of the parthenogenetic treatments. The assumption of neutrality in the sexual progeny can be tested indirectly by homogeneity tests among control crosses since under neutrality the perturbation level of the SO female parent and the genotype of the marker males used in the crosses should have no effect on the genotype frequencies of their progeny. Homogeneity between the male and female progeny from a single control cross is also expected under neutrality. Since the controls are used to estimate recombination frequencies between linked markers, homogeneity tests were conducted on the autosomal complex of *A*, *B*, *v* and *X* and on the *X*-linked complex of *G* and *sl*. The results obtained using unlinked pairs of these markers will be discussed later.

Homogeneity is first tested by regarding each control mating type as a distinct category and, wherever possible, the male progeny within a cross as distinct categories. Each control category is partitioned into the respective recombination categories for the *X*-linked systems (parental and recombinant) and for the autosomal system (small numbers necessitated the pooling of the triple crossover category with the double crossover category with recombination between *A* and *B* and between them and *X*). There homogeneity χ^2 across control mating types and sexes for the autosomal system was 85.96 with 66 degrees of freedom (not significant at the 5% level). The homogeneity for the *X*-linked system was 23.18 with 11 degrees of freedom (significant at the 5% level). Therefore, by the homogeneity criteria, the autosomal markers fit the prediction of neutrality while the *X*-chromosome markers do not. Further analysis showed that the *X*-linked markers lack homogeneity between the sexes. The frequency of O and S parental-type *X* chromosomes is .559 in the control females and .615 in the males. The male value is similar to the parthenogenetic frequencies of .614 in the 100% experiment and .636 in the 60% treatment. As will be discussed later, we feel these results indicate that some sort of selection is occurring on the *X*-linked markers of the males. To avoid biasing our analysis of the *X*-linked markers, the recombination frequency between *G* and *sl* was estimated using female controls only.

A direct test of our assumption of neutrality in the controls is to compare the observed recombination frequency for unlinked pairs of markers with the *a priori* expected frequency of .5. There are eight such systems, and each is tested by the statistic $u = (\hat{p} - 0.5)/\sqrt{.25/n}$, where n is the sample size and p is the observed frequency of parental genotypes in the sample. Under the hypothesis that $p = 0.5$, u is asymptotically normally distributed with a mean of zero and variance of one. The results are given in Table 4, as are the results for the two parthenogenetic treatments that had unlinked pairs. Although the eight tests for the controls are not independent, not one is significant at the 5% level and the numbers of positive and negative deviations are approximately equal (5:3). Therefore, with respect

TABLE 4

Deviations of observed proportion of parental genotypes ($\hat{p}$) *from 0.5 for unlinked pairs of markers*

Markers	Control progeny		Parthenogenetic progeny			
			100% Perturbation		60% Perturbation	
	$\hat{p}$	u‡	$\hat{p}$	u	$\hat{p}$	u
A-G	.4971	—0.329	.5606	1.969*	.5159	0.799
B-G	.5105	1.181	.5584	1.904†	.5104	0.520
v-G	.5060	0.624	.5571	1.883†	.4888	—0.559
X-G	.4925	—0.701	.5413	1.285	.4919	—0.380
A-sl	.5018	0.251	.5451	1.561	.5176	1.010
B-sl	.5043	0.581	.5657	2.294*	.5066	0.383
v-sl	.4877	—1.562	.5942	3.334***	.4951	—0.277
X-sl	.5131	1.276	.5148	0.486	.5013	0.074

* Significantly different from 0.5 at 5% level.
*** Significantly different from 0.5 at 0.1% level.
† Significantly in excess of 0.5 at 5% level. (All systems that are significantly different are also significantly in excess.)
‡ $u = (\hat{p} - 0.5) \div \sqrt{0.25/n}$, n = sample size.

to those systems that may be directly tested, we fail to reject neutrality in the controls.

The controls are used to estimate the expected frequencies of the genotypes for the linked two-locus and all of the three-locus marker combinations studied in the parthenogenetic flies. The proportional sampling error ($s_p/\hat{p} \times 100$) for the two-locus systems ranges from a low of 0.8% to a high of 3.4% and for the three-locus systems from 0.5% to 4.8%. As will be shown below, the proportional differences in viabilities between genotypes often runs from one to two orders of magnitude greater than these proportional errors. Because the selective forces detected are so large, error due to sampling in the controls will have at most only a minor quantitative effect on the evaluation of fitness and will not alter the inferences based on the qualitative patterns we report below.

Parthenogenetic progeny

There are many lines of evidence that bear on our hypothesis that the S and O genomes are adapted to total homozygosity. The first of these is simply the number of viable adult offspring produced parthenogenetically by the females at the three levels of perturbation. When 100% of the genome was subject to perturbation, the SO_1 females produced an average ± standard deviation of 1.63 ± 1.10 offspring per female; at the 60% perturbation level, 5.36 ± 1.11; and at the 40% level, 10.25 ± 1.44 (pooled across SO_3 and SO'_3 parents). The reduction in number of parthenogenetic progeny per female as perturbation level increases indicates that the more the S and O genomes are perturbed away from their original state by meiosis, the lower the absolute fitness of the perturbed genotypes. This also implies that very strong selective forces could be operating among the surviving genotypes of the 100% perturbation level.

The genotypes of the survivors range from the S and O parental genotypes to highly recombinant ones. A coadaptation hypothesis would predict that S and O

parental genotypes should have a selective advantage over the recombinants in the totally homozygous genetic environment but not in the heterozygous genetic environment. The difference between the controls and the parthenogenetic progeny in the proportions of parental genotypes is tested with the statistic: $d = (p_e - p_c)/\sqrt{\bar{p}(1-\bar{p})\,(1/n_c + 1/n_e)}$, where p_c is the frequency of parental types in the controls in a sample of size n_c, $p_{\bar{e}}$ the frequency in a particular parthenogenetic treatment level in a sample of size n_e and $\bar{p} = (n_c p_c + n_e p_e)/(n_c + n_{\bar{e}})$. Under the hypothesis $p_{\bar{e}} = p_c$, d is asymptotically normal zero, one. A d value outside the range -1.96 to $+1.96$ would indicate a significant *difference* at the 5% level, and a d greater than 1.645 indicates a significant *excess* at the 5% level of parental types in the experimental group over that in the controls. The results of this test for all two- through six-locus combinations of the markers are summarized in Table 5 (a more detailed analysis is available upon request). The

TABLE 5

Summary of the test results using all two- through six-locus systems which compare the frequencies of parental-type marker combinations in the various parthenogenetic treatment groups with those in the sexual controls

No. of loci	Linkage relationship	Perturbation level: 100% No. of systems	100% No. with significant excess*	100% No. with significant difference†	60% No. of sys.	60% No. sig. exc.	60% No. sig. diff.	40% No. of sys.	40% No. sig. exc.	40% No. sig. diff.
2	1 2	3	0	0	3	3	3	2	1	0
	1 2	4	4	3	4	0	0	4	0	0
	1 2	8	5	3	8	0	0	0	–	–
3	1 2 3	4	4	4	4	1	0	4	2	2
	1 2 3	8	8	6	8	2	1	0	–	–
	1 2 3	8	7	7	8	0	0	0	–	–
4	1 2 3 4	1	1	1	1	1	1	1	1	1
	1 2 3 4	2	2	2	2	2	2	0	–	–
	1 2 3 4	4	4	4	4	0	0	0	–	–
	1 2 3 4	8	8	8	8	0	0	0	–	–
5	1 2 3 4 5	2	2	2	2	0	0	0	–	–
	1 2 3 4 5	4	4	4	4	2	1	0	–	–
6	1 2 3 4 5 6	1	0	0	1	0	0	0	–	–

* The "d-statistic" is regarded as a one-tailed test at the 5% level of significance.
† The "d-statistic" is regarded as a two-tailed test at the 5% level of significance.

parthenogenetic progeny of the SO_3 and SO'_3 females are pooled for this and all subsequent analyses since homogeneity was not rejected for any of their marker combinations. The absolute number of significant results given in these tables must be interpreted with caution because the tests are obviously correlated. However, the deviations from the controls are often so large that the primary problem is one of interpretation of the effect and not whether it is present. Hence, the *pattern* of test results with respect to number of markers, linkage relationships and perturbation level becomes the important feature of the data.

The analyses of the 2–6-locus systems reveal selection favoring parental genotypes among the survivors at the 100% perturbation level. Nine out of fifteen two-locus combinations show a significant excess of S and 0 parental genotypes and, although not shown, all pairs show some excess. Since this test is insensitive to additive selection, these results imply the presence of strong nonadditive selection operating upon the 100% perturbed parthenogenetic progeny that involves both the linked and unlinked marked chromosome segments. For the 3- through 6-locus combinations, the same pattern holds; all combinations show an excess of parental genotypes in the 100% experiment. Furthermore, these excess tend to become larger as the number of loci in the system increases. Thus, 3 out of 15 of the two-locus systems are significantly different at the 1% level, while 14 out of 20 of the three-locus systems and all of the 4- and 5-locus systems are significant at the 1% level. In fact, 5 out of 6 5-locus systems are significant at the 0.01% level. If the O and S genomes are coadapted to total homozygosity and this coadaptation involves non-additive interactions between loci scattered throughout the genome, the deviations from the controls should increase as the number of markers in the system increases since the d statistic is being influenced by a greater proportion of the total genome. In other words, a fly "parental-type" for three markers will, on the average, have more of the total genome "parental-type" than a fly "parental-type" for only two markers. This prediction must be tempered with caution because as the number of markers increases, both $\bar{p}$ and $n_c + n_e$ decrease, causing a leveling off or a decline of the d values. Such a sampling effect could explain the fact that the 6-locus system shows no significant parental excess in the 100% treatment, although it still shows an excess over the controls. The magnitude of the d statistics would also decline if the markers used extend beyond the level of genetic organization that behaves as a non-additive unit. Such an effect is perhaps seen in 60% and 40% treatments, as will now be discussed.

Table 5 shows that for the 60% and 40% perturbation levels all significant excesses of parental genotypes in the parthenogenetic progeny over the controls are restricted to systems having markers on the same chromosome arm. There are no significant excesses of parental types for any two-locus system involving unlinked pairs (see Table 4) or pairs on the same chromosome but different arms. Furthermore, within this latter group of non-significant systems there are about the same number of positive and negative deviations from the controls—a marked contrast from the uniform excesses seen in the 100% perturbation results. The statistical significance of parental genotype excesses tends to increase in the

100% treatment as the number of loci in the system increases, but this *pattern* is altered in the 60% and 40% treatments. All significant excesses are restricted to those combinations involving markers on the same arm, and the more markers there are on different arms, the smaller the deviation from the controls. Selection is operating in a non-additive fashion only on markers on the same chromosome arm in the 60% and 40% treatments, and either no selection or additive selection exists between arms for these treatment levels.

Unfortunately, the "*d*" test does not allow discrimination between a hypothesis of no selection and one of additive selection. Even when the presence of non-additive selection is indicated, *d* gives us no insight into underlying models of fitness that might give an adequate description. To circumvent these problems, we estimated the fitnesses of the 1, 2- and 3-locus genotypes and evaluated the ability of alternate selection models as descriptors of the observations using the techniques given in the statistical methods section.

The fitness analyses for the one-locus systems are given in Table 6. There is a fairly consistent trend for the fitness of the S-type alleles to increase relative to the O-type as perturbation level decreases. The perturbation level is decreased by increasing the portion of the genome fixed for S-type chromosomes. Under a coadaptation hypothesis we would expect the marginal S-allele fitnesses to increase as the background becomes more S-type, which is what we observe.

The two-locus fitness analyses also support the coadaptation hypothesis. The 100% results (Table 7) show that all 2-locus combinations have a positive interaction term which is usually larger in magnitude than the additive effects. Only the two visible markers, *sl* and *v*, give additive effects greater in magnitude than the interaction term. Seven of the 2-locus systems show significant selection, but only two could be adequately predicted by an additive and/or multiplicative model. The non-additive and non-multiplicative systems involve both linked and unlinked marker pairs. Finally, a comparison of the numerical magnitudes of the test of unrestricted selection *vs.* the null model and the tests for the goodness of fit of the additive and multiplicative models gives an indication of the importance

TABLE 6

The one-locus fitness analysis of the parthenogenetic treatment groups

	Perturbation level								
	100%			60%			40%		
Marker	Fitness of O-allele	Fitness of S allele	Test of selection *vs.* H_0 (d.f.=1)	Fitness of O allele	Fitness of S allele	Test of H_0	Fitness of O allele	Fitness of S allele	Test of H_0
B	1.000	0.949	0.211	1.000	1.158	4.471*	1.000	1.025	0.342
A	1.000	0.935	0.333	1.000	0.956	0.432	1.000	1.063	2.151
v	1.000	0.809	3.486	1.000	0.833	6.952**	1.000	0.914	4.719*
X	1.000	0.929	0.370	1.000	0.832	6.150*	1.000	0.967	0.162
sl	1.000	0.754	6.186*	1.000	0.908	1.924	——	——	——
G	1.000	1.100	0.619	1.000	0.842	4.634*	——	——	——

* The null hypothesis of no selection is rejected at the 5% level.
** The null hypothesis of no selection is rejected at the 1% level.

TABLE 7

The two-locus fitness analysis of the 100% perturbation parthenogenetic treatment group

Markers	Unrestricted fitness components				Test of unrestricted fitness *vs.* H_0 (df=3)	Goodness of fit tests of	
	μ	α	β	ε		Additive model (df=1)	Multiplicative model (df=1)
(*B A*) —●	.902	.015	.027	.055	1.281	0.824	0.812
(*v X*) —●	.844	.070	—.003	.089	3.872	2.203	2.229
(*sl G*) —●	.835	—.052	.124	.093	10.273*	3.250	3.924*
A v —●—	.798	.017	.077	.108	8.159*	5.320*	5.207*
A X —●—	.837	.029	.029	.106	4.584	3.973*	3.909*
B v —●—	.742	.018	.066	.174	18.780***	16.367***	16.196***
B X —●—	.774	.046	.026	.155	11.252*	10.154*	9.998*
A G —● ●—	.917	.014	—.042	.111	4.548	3.888*	3.942*
sl A —● ●—	.787	.118	.024	.071	9.323*	2.441	2.254
G B —● ●—	.907	—.044	.031	.106	4.697	3.634	3.753
sl B —● ●—	.768	.111	.020	.101	11.685*	5.278*	5.084*
G v —● ●—	.868	—.035	.067	.099	5.859	3.553	3.783
sl v —● ●—	.699	.096	.074	.132	19.203***	11.188***	9.791***
G X —● ●—	.903	.000	.022	.075	1.804	1.654	1.655
sl X —● ●—	.823	.122	.030	.024	6.479	0.237	0.161

* Significant at the 5% level.
*** Significant at the 0.1% level.

of the non-additive and non-multiplicative epistatic effects in determining the two-locus outcomes. If all of these tests are approximately equal in numerical size, then such epistasis is of overwhelming importance. If the goodness of fit tests are around half the size of the test of the unrestricted model *vs.* the null hypothesis, both epistatic and additive/multiplicative effects are important. If the goodness of fit tests are much smaller than those of the unrestricted model *vs.* the null model, epistasis does not play an important role. Of the 15 combinations of marker pairs in the 100% perturbation experiment, only the unlinked system of *sl*/*X* demonstrated this last pattern.

These conclusions are confirmed in an even stronger fashion by the three-locus analyses of the 100% experiment. Table 8 shows that 13 out of 20 three-locus combinations have significant selection, and in only 3 of these cases do some subset of the markers define an additive or multiplicative unit. There is a consistent pattern in which most of the goodness of fit tests are at least within 1/3 of

TABLE 8

Test results of the three-locus fitness analysis of the 100% perturbation parthenogenetic treatment group

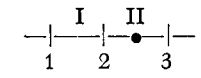
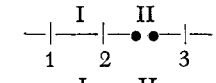
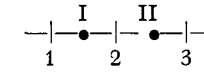
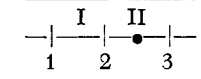
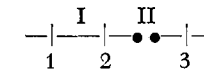
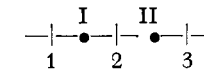
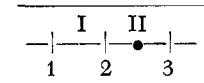
Markers	Test of unrestricted fitness *vs.* H_0 df=7	Goodness of fit tests of fitness models: Locus additive df=4	Locus multiplicative df=4	Chromosome† additive df=3	Chromosome multiplicative df=3
(*B A*) *X*	12.512	11.220*	11.091*	10.465*	10.341*
(*B A*) *v*	20.199**	17.699**	17.445**	17.382***	16.990***
(*v X*) *A*	8.899	7.470	7.381	5.020	4.865
(*v X*) *B*	21.784**	20.028***	19.700***	17.813***	17.285***
(*B A*) *G*	7.510	6.635	6.739	5.896	5.920
(*B A*) *sl*	15.014*	8.059	7.701	7.615	7.023
(*v X*) *G*	5.147	4.172	4.190	3.248	3.249
(*v X*) *sl*	19.679**	13.217*	12.362*	11.162*	9.912*
(*sl G*) *A*	18.491**	12.007*	12.482*	9.694*	9.647*
(*sl G*) *B*	23.575**	16.628**	16.922**	14.585**	14.280**
(*sl G*) *X*	15.030*	9.884*	10.019*	6.878	6.730
(*sl G*) *v*	33.865****	26.568****	26.276****	23.501****	22.116****
B X *G*	18.362*	16.761**	16.705**	9.275*	9.663*
B X *sl*	20.234**	14.075**	13.449**	4.137	3.541
B v *G*	28.505***	26.360****	26.433****	9.682*	11.187*
B v *sl*	39.224****	31.944****	30.516****	16.499***	14.320**
A X *G*	6.496	6.131	6.120	5.220	5.239
A X *sl*	11.228	5.261	4.982	1.306	1.324
A v *G*	12.209	10.171*	10.412*	7.299	7.676
A v *sl*	27.803***	19.641***	18.047**	14.170**	12.522**

† For the first 12 systems, "chromosome" really means "chromosome arm".
* Significant at the 5% level.
** Significant at the 1% level.
*** Significant at the 0.1% level.
**** Significant at the 0.01% level.

the size of the test of unrestricted selection *vs.* the null hypothesis. The results for the 100% level in Table 9 show that recombination between either same-arm, same-chromosome or unlinked markers all cause drastic fitness reductions, and the simultaneous occurrence of recombination between linked markers and assortment between unlinked ones causes a further fitness decline. This fitness reduction is greater in magnitude than that for the two-locus case, indicating the detection of more selection with three loci than with two. Also, there is a tendency for the double recombinant class to gain in fitness over the lowest fitness for a

TABLE 9

Average† fitness patterns for the three-locus genotypes of the parthenogenetic treatment groups

Perturbation level	Linkage of markers	Fitness of genotype: Parental	Rec. I	Rec. II	Rec. I & II	Number of systems averaged
100%		1.000	0.669	0.642	0.768	4
		1.000	0.749	0.704	0.680	8
		1.000	0.627	0.695	0.614	8
					Total	20
60%		1.000	0.770	1.007	0.778	4
		1.000	0.762	0.972	0.742	8
		1.000	0.950	0.974	1.045	8
					Total	20
40%		1.000	0.898	0.955	0.829	4

† The average is taken over systems which share the same basic linkage relationships and over the parallel meiotic classes. Averaging over meiotic classes eliminates any additive effects.

single recombinant class only in those combinations involving three linked loci (whenever the double recombinant class represents a true double crossover class). This is easily explicable if there are interacting loci, in addition to the markers, scattered throughout the chromosome, since the second crossover "undoes" the first to yield a chromosome which on the average is more S or O parental-type than a single crossover chromosome. Therefore, other loci linked to the markers seem to be involved in the coadaptation.

The qualitative differences between perturbation levels seen in the pattern of the *d* statistic results are further illustrated by the detailed fitness analyses of the two- and three-locus combinations. Table 10 shows that all three of the two-locus, same-arm pairs have statistically significant selection in the 60% treatment, and in all cases the models that assume the individual markers are additive/multiplicative units are rejected. Seven of the systems involving markers on different arms also show significant selection for the 60% treatment, but all are adequately explained by the additive or multiplicative model. However, the most impressive evidence is simply the consistency of the patterns one observes when contrasting the numerical size of the test of the unrestricted model *vs.* the null hypothesis to that of the goodness of fit tests for the additive/multiplicative models. As pre-

TABLE 10

The two-locus fitness analysis of the 60% and 40% perturbation parthenogenetic treatment groups

Perturbation level	Markers	Unrestricted fitness components μ	α	β	ε	Test of unrestricted fitness *vs.* H_0 (df=3)	Goodness of fit tests of: Additive model (df=1)	Multiplicative model (df=1)
60%	(*B A*)	.938	.074	—.107	.096	15.632***	6.276*	7.965**
	(*v X*)	.801	.073	.038	.088	15.680***	6.521*	6.089*
	(*sl G*)	.778	.058	.041	.122	19.865***	14.833***	14.206***
	A v	.930	—.001	.091	.021	8.416*	0.428	0.431
	A X	.891	.008	.085	.016	7.039	0.225	0.206
	B v	.943	—.062	.088	.030	11.252*	0.857	1.228
	B X	.999	—.083	.101	—.017	11.238*	0.208	0.055
	A G	.860	.038	.074	.027	6.415	0.639	0.498
	sl A	.910	.050	.008	.032	3.519	1.022	0.998
	G B	.933	.076	—.028	.019	5.101	0.271	0.342
	sl B	.991	.054	—.059	.013	5.623	0.147	0.229
	G X	.827	.096	.090	—.013	14.720***	0.144	0.475
	sl v	.885	.042	.081	—.008	9.040*	0.076	0.164
	G v	.828	.066	.124	—.018	18.927***	0.313	0.761
	sl X	.860	.064	.073	.002	9.330*	0.005	0.009
40%	(*B A*)	.940	—.034	.007	.037	4.644	2.439	2.472
	(*v X*)	.927	.049	—.029	.053	9.907*	5.103*	5.419*
	A v	.996	—.035	.046	—.007	7.046	0.108	0.063
	A X	.985	—.027	—.002	.043	5.365	3.878*	3.873*
	B v	.958	—.014	.041	.015	5.136	0.551	0.599
	B X	.991	—.017	—.005	.030	2.550	1.915	1.906

viously explained, this pattern yields information on the relative importance of non-additive/non-multiplicative selection *vs.* the additive or multiplicative components. The pattern clearly demonstrates that individual marker loci—whether or not they are on the same arm—do not form units of selection in the 100% treatment. The pattern for the 60% experiments is also clear—all epistatic interactions are confined to markers on the same arm with none between arms. The 60% pattern is reflected, though quantitatively less strongly, in the 40% treatment. Thus, the *pattern* of fitnesses changes with perturbation level.

This pattern is also seen in the three-locus test results for the 60% and 40% treatments given in Table 11. For the 12 combinations with markers on the same

TABLE 11

Test results of the three-locus fitness analysis of the 60% and 40% perturbation parthenogenetic treatment groups

Perturbation levels	Markers	Test of unrestricted fitness *vs.* H_0 d.f.=7	Goodness of fit tests of fitness models			
			Locus additive d.f.=4	Locus multiplicative d.f.=4	Chromosome† additive d.f.=3	Chromosome multiplicative d.f.=3
	(*BA*) *X*	29.061***	14.515**	15.569**	2.014	0.960
	(*BA*) *v*	23.739**	10.689*	12.102*	2.358	3.097
	(*vX*) *A*	19.551**	9.141	8.625	1.710	1.715
	(*vX*) *B*	30.436****	16.766**	16.833**	10.578*	9.923*
	(*BA*) *G*	13.568	5.544	6.076	0.720	0.685
	(*BA*) *sl*	19.307**	11.672*	12.406*	1.616	1.813
	(*vX*) *G*	27.985***	6.697	6.687	0.713	1.175
	(*vX*) *sl*	21.470**	7.998	7.835	1.053	1.146
	(*sl G*) *A*	21.563**	15.682**	14.941**	2.111	1.722
	(*sl G*) *B*	20.164**	14.933**	14.402**	0.685	0.693
	(*sl G*) *X*	32.410****	15.712**	14.680**	4.889	3.786
	(*sl G*) *v*	35.636****	16.049**	15.809**	2.524	1.602
60%						
	B X G	16.632*	1.450	1.902	1.171	1.728
	B X sl	15.488**	1.064	1.301	1.031	1.297
	B v Sl	19.749**	1.331	2.047	1.330	2.013
	B v sl	15.006*	1.866	2.513	0.920	1.285
	A X G	16.488*	1.110	1.289	1.103	1.289
	A X sl	12.247	1.488	1.511	1.413	1.477
	A v G	22.137**	2.156	2.845	1.080	1.628
	A v sl	13.732	3.115	3.284	2.895	2.991
	(*BA*) *X*	20.010**	18.522***	18.503***	2.710	2.706
	(*BA*) *v*	9.297	2.271	2.199	1.365	1.348
40%	(*vX*) *A*	19.775**	14.021**	14.153**	6.821	6.709
	(*vX*) *B*	15.540*	10.692*	10.955*	4.761	4.787

† For the first twelve systems and all the 40% systems, "chromosome" really means chromosome arm.

arm in the 60% level, 11 show statistically significant selection. Of these 11, only 1 shows a fitness pattern that cannot be explained by assuming that markers on the same arm form additive/multiplicative units of selection. Moreover, the single exception (*v-X-B*) has the same pattern of relative test sizes that the other 11 show. The negative of the difference in goodness of fit between the model considering the individual markers as additive/multiplicative units and the model considering chromosome arms as additive/multiplicative units is distributed as a chi-square with 1 degree of freedom. Table 11 reveals that for all 12 marker combinations in the 60% treatment that involve a same arm pair, there is a significant increase in goodness of fit of the model with chromosome arms as the unit of selection over that with the individual marker loci as the unit of selection. This outcome is in marked contrast to the 100% results where both the individual markers and the chromosome arms as units of selection were about equally poor descriptors of the outcome of selection. For the eight combinations with no same-arm markers, the test patterns are radically different in the 60% case. Invariably, the goodness of fit of the individual markers as additive/multiplicative units of selection is excellent and is not significantly improved by grouping the two markers on the same chromosome but different arms into a unit of selection. This is also borne out by the fitness patterns given in Table 9. The only type of recombination that drastically reduces fitness is recombination between markers on the same arm. Thus, the fitnesses of the Rec II class are now uniformly high and the double crossover effect seen with three linked loci in the 100% treatment has vanished. When there are no markers on the same arm, all fitnesses are high and selection is explicable entirely in terms of the marginal additive effects of individual markers. The 40% results are similar, with three out of the four combinations showing significant selection that can be explained by assuming chromosome arms are the additive/multiplicative units of selection.

DISCUSSION

"Genes are said to be coadapted if high fitness depends upon specific interactions between them" (WALLACE 1968). Using this definition of coadaptation, our experimental results indicate that the O and S genomes are coadapted under conditions of total homozygosity. Both of these genomes display high fitness when intact, but this fitness is greatly decreased by meiotic perturbation. Furthermore, the detailed fitness analyses indicate the observed fitness effects are primarily due to non-additive and non-multiplicative interactions between chromosome segments scattered throughout the genome. These interactions occur between all marked segments in the 100% perturbation experiment, and the 60% and 40% experiments indicate the fitness effects observed on the *X* and metacentric chromosomes are strongly influenced by the state of the remaining autosomes.

The idea of "coadaptation" is not a new one. DOBZHANSKY (1948, 1950) originally used the word coadaptation to refer to the maintenance of chromosomal polymorphism in local populations since his results indicated that chromo-

somes with different gene arrangements that occur in the same geographic area are coadapted such that heterozygotes show high fitness. This is a rather restrictive definition of coadaptation, unlike WALLACE's, and cannot be applied directly to our analysis since there are no inversion differences between the O and S stocks, and since we were concerned with the fitness of homozygotes rather than heterozygotes. However, a much broader definition of coadaptation is to regard it as an integration of the local gene pool, as illustrated by the experiments of BRNCIC (1954). BRNCIC used crosses both within and between geographical strains and various backcrosses to breed four basic types of flies: (1) those carrying two intact chromosomes from one locality, (2) those carrying two intact chromosomes, one from each of two localities, (3) those carrying one intact and one inter-locality recombinant chromosomes, and lastly (4) those carrying two inter-locality recombinant chromosomes. He detected inhomogeneities in the frequencies of these types of flies relative to a marked standard and showed that the fitness order is: (2) > (1) > (3) > (4). Hence, flies bearing the most recombinant chromosomes had the lowest fitness.

Although BRNCIC's experiments were not carried out in a totally homozygous genetic environment, we see the same basic pattern in our work—the more recombinant the genotype, the lower the fitness. Since the O and S stocks come from different geographical localities, the possibility exists that the coadaptation we observed is a geographical coadaptation similar to what BRNCIC studied and really has nothing to do with parthenogenesis or its genetic consequences *per se*. We must return to the control crosses for information on this possibility. As can be seen from Table 2, some of the control crosses represent backcrosses to an "intact" geographical strain (C_1, C_2, C_4, C_6), while others represent crosses to strains with "mixed"-origin chromosomes (C_3, C_5, C_7, C_8, C_9). If our results are due to geographical coadaptation, we should expect to observe inhomogeneities in the control genotype frequencies similar to BRNCIC's results; yet, with the exception of the *X*-linked systems, no such inhomogeneities were observed. Furthermore, the inhomogeneity of the *X*-linked markers was *not* between types of control crosses, but rather was between the sexes *within* a cross. Consequently, the evidence indicates we are not dealing with a geographical coadaptation in the classical sense, but rather with a coadaptation for a specific genetic environment —total homozygosity. Therefore, the S and O stocks have genomes with an allele content coadapted to maintain high fitness under total homozygosity, and when this genetic environment is modified these fitness effects vanish. The lack of evidence for geographical coadaptation under the heterozygous genetic environment is not entirely surprising. First, geographical coadaptation is not universally observed even in sexual strains of Drosophila (MCFARQUHAR and ROBERTSON 1963; RICHARDSON and KOJIMA 1965; SINGH 1972). Secondly, at the time of the experiment the O and S stocks had been reproducing parthenogenetically for approximately 150 and 240 generations, respectively. Furthermore, each had been derived from lab stocks which had experienced some inbreeding. Even if there had been some geographical coadaptation in the natural populations inhabiting Salvador and Oahu, there is no reason to expect it to be preserved

after so many generations of evolution under a radically different set of genetic conditions.

Further evidence that we are dealing with an adaptation to a genetic environment is provided by a closer look at the inhomogeneity between control males and females for the *X*-linked markers. Both the parthenogenetic females and control males receive only one *X* chromosome with no opportunity for heterozygosity at *X*-linked loci. On the other hand, the control females have a pair of *X*'s with potentially a large percentage of heterozygous loci. The frequencies of *X*-linked marker genotypes in control males are homogeneous with those from the parthenogenetic data and were significantly different from those of their sexually produced sisters. However, the marker systems on the autosomes, which are heterozygous in both control males and females, are homogeneous between sexes but different from the parthenogenetic results. This contrast between the behavior of *X*-linked *vs.* autosomal markers in males indicates that the selection is associated with the nature of the genetic environment and not mode of reproduction *per se*.

Placing the emphasis on the nature of the genetic environment, we can draw even more parallels between this study and previous work on "synthetic lethals" (e.g., WALLACE *et al.* 1953; DE MAGALHAES, DE TOLEDO and DA CUNHA 1965). In these experiments, dominant markers and inversions were used to extract chromosomes from natural populations of Drosophila and then make these chromosomes homozygous. Two different chromosomes that were non-lethal when homozygous were then allowed to recombine, and lethals were recovered in higher-than-expected frequencies. In fact, synthetic semi-lethals can arise from the action of two different chromosome pairs (DOBZHANSKY, SPASSKY and ANDERSON 1965). Such synthetic lethals must arise due to deleterious interactions between non-alleles because single-locus lethality is excluded by the homozygous effects of the non-recombinant chromosomes. Furthermore, the alleles are specifically tested for their interactions under homozygosity. These results are very similar to ours and give independent confirmation of the importance of non-allelic interactions under total homozygosity. It is also interesting to note that HILDRETH (1956) could not find any synthetic lethals in the *X* chromosome of *D. melanogaster*. The reason for this is probably the same as our explanation for the inhomogeneity of the *X*-linked markers' behavior in control males *vs.* females —that hemizygosity mimics the genetic environment of total homozygosity. Consequently, selection acting through males in natural populations should eliminate *X* chromosomes with deleterious combinations of genes under hemizygosity and thus indirectly under homozygosity.

We may now return to DOBZHANSKY's original use of the word coadaptation to explain heterosis and show that in a very fundamental sense it is consistent with our usage of the word to explain high fitness under homozygosity. The flies DOBZHANSKY studied are sexually reproducing and thus have a mode of reproduction that can produce much heterozygosity; the parthenogenetic strains we studied are characterized by total homozygosity. In both cases, we can say that the genes are coadapted to yield high fitness in the genetic environment under

which they are normally expressed, a result well known to plant breeders (e.g., MOLL and STUBER 1971). This definition of coadaptation is consistent with CROW's (1957) concept of alleles being "tested" by natural selection for different types of fitness effects as a function of mode of reproduction. Further evidence for this idea comes from recent work by TEMPLETON, CARSON and SING (1975) on the parthenogenetic capacity of females drawn from sexually reproducing populations of mercatorum. This work shows that females drawn from natural, bisexual populations characterized by high levels of heterozygosity are *more* capable of producing parthenogenetic progeny than the average female drawn from an inbred, bisexual strain. Furthermore, within the group of bisexual females capable of parthenogenesis, the level of heterozygosity is higher than in those incapable of parthenogenesis. These results are exactly the opposite of the results reported here in which parthenogenetic capacity decreases (10.25 progeny/female $\rightarrow$ 1.63) as heterozygosity increases ($SO_3 \rightarrow SO_1$). The contradiction is resolved when we consider that the genomes being challenged to develop parthenogenetically in these two cases had previously evolved under very different genetic environments. The bisexual stocks had evolved with a mode of reproduction that effectively excludes the possibility of total homozygosity, and hence the genes would not be coadapted to this genetic environment. The chances of any bisexual female producing a genome-type that would do well in this radically different state of total homozygosity should be quite small, and the probability of including at least one of these rare genomes among a particular bisexual female's meiotic products is proportional to the genetic diversity among her meiotic products. This last factor is a function of her level of heterozygosity under pronuclear duplication; hence, the greater the heterozygosity, the greater the chance of a parthenogenetic progeny. On the other hand, if the genomes had previously been coadapted to total homozygosity, the chance decreases of including a coadapted genome among a female's meiotic products as her level of heterozygosity increases. These observations illustrate the importance of the genetic state under which genes evolve in creating coadapted complexes.

As shown above, our work bears many similarities to previous work done on coadaptation and synthetic lethals. However, there are some major differences between our experimental design and the previous ones that allow us to attack more directly the problem of the unit of selection. One limitation of the previously cited studies is that they treat the genome or chromosome as a black box and have no handle on genotype organization within the array of surviving genotypes. Much of their data is somewhat analogous to our data on the number of parthenogenetic progeny produced per female at each perturbation level. Such data indicates the presence of selection, but gives little insight into the unit of selection that explains the genetic outcome of the surviving population. However, our conclusions are based primarily on the relative viabilities associated with certain marker systems observed among the surviving parthenogenetic progeny. This aspect of our experimental design separates the present study from previous coadaptation studies and allows us to draw conclusions about the unit of selection. The most obvious conclusion we may draw about the unit of selection in the

populations we studied is that it is a function of perturbation level. At the 100% perturbation level the only meaningful unit of selection we could define was the entire marked segment of the genome. At the 60% and 40% levels the outcome of selection could be very accurately described using markers on the same chromosome arms as units of selection. The exact meaning of this dependency upon perturbation level is unclear at present, but one possible interpretation is as follows. One can regard the perturbation level as an indication of the intensity of selection, either in terms of the absolute number of parthenogenetic progeny surviving at a given perturbation level or in terms of the relative fitness differences between parental and recombinant genotypes within the survivors at a given level. If this is true, we can conclude that the unit of selection is a function in part of the intensity of selection: the more intense the selection, the more the whole genome tends to hold together as a unit.

Another, but not necessarily incompatible, interpretation of the dependence of the unit of selection upon perturbation level rests upon our definition of the unit of selection as a level of genetic organization to which fitness measures can be ascribed that combine additively or multiplicatively to describe the outcome of selection in a population. Note that "fitness" here is used in describing a population and not an individual—that is, the "unit of selection" is an evolutionary measure and *not* a measure of an individual's degree of adaptiveness. Since selection can only operate upon the genotypes actually manifested in a zygotic pool, the outcome of selection—and hence the unit of selection—is influenced not only by individual fitness differences, but by all other factors that affect the spectrum of realized zygotic genotypes. In our experiments the perturbation level would greatly modify the nature of the zygotic pool upon which selection would operate, and this could explain the differences in the observed unit of selection. Theoretical support for this view comes from some computor simulations (WILLS, CRENSHAW and VITALE 1970; FRANKLIN and LEWONTIN 1970). These studies defined precisely how the effects of non-alleles combine in determining an individual's fitness. A variety of models were used, including those which combine the fitness effects of a given locus in multiplicative and in "threshold" fashions with other allelic effects in determining the fitness of an individual. However, the formulation took into account an interaction between close linkage and selection. Both studies detected large deviations from the predictions of the genetic load theory, which treats each locus as an independent multiplicative unit of selection. That is, some "unit of selection" greater than the individual allele was necessary to describe the micro-evolutionary processes that were simulated. Furthermore, both the multiplicative individual fitness model and the threshold individual fitness model yield essentially the same results. This robustness to varied fitness models probably occurs because the outcome of selection depends not only on the nature of the selective forces operating upon individuals but also on the actual genotypes present in the population on which selection is operating. In these simulations close linkage greatly influenced this latter factor and was apparently of overriding importance in determining the outcome of selection.

Such a result illustrates the important distinction between how loci interact to create an individual's fitness and the unit of selection, which describes the genetic outcome of selection in a population.

Besides perturbation level, physical linkage also seems to be an important factor in determining the unit of selection. The basic fitness patterns are remarkably consistent when the markers are grouped according to the type of physical linkage relationships between them. Strong epistatic forces are always observed between markers on the same arm, regardless of perturbation level. Furthermore, the observation that true double crossover genotypes recover high fitness only in the 100% perturbation experiment illustrates an interesting interaction between linkage and perturbation level. Most importantly, the two- and three-locus fitness analyses indicate that both the S- and O-type chromosome arms have a higher fitness than the SO recombinants even in the 60% and 40% treatments. This occurs despite a predominantly S-type background at those levels and the fact that the recombinant arms would bear more S-type alleles on the average than an O arm. Thus, selection under a broad range of conditions seems to preferentially operate upon linked blocks of genes, a result not entirely unexpected from theoretical considerations (WILLS, CRENSHAW and VITALE 1970; FRANKLIN and LEWONTIN 1970; SLATKIN 1972).

Our final conclusion concerning the unit of selection is actually more of a warning. It is that selective neutrality may arise as an artifact in a coadapted genetic complex if the genetic markers used do not identify the unit of selection. The strongest selection detected at a multi-locus level is observed in the 100% treatment, yet only one of six markers (the visible marker *sl*) shows any statistically significant marginal selection in the one-locus fitness analyses (Table 6). These data illustrate that loci may appear neutral on the margin but still be involved in a highly selected gene complex. This observation is further documented by data from the multi-locus combinations. In the 60% perturbation, the unit of selection is the chromosome arm. However, when a two- or three-locus system is studied with no two markers on the same arm, very little selection is detected as compared to the situation when two markers were on the same arm (Table 9 or 10). Once again, the interpretation of neutrality was implied only because the markers did not identify the unit of selection.

Whether these conclusions can be generalized beyond the two populations we have so far studied remains to be seen, although some on-going and planned experiments should shed light on this. What these experiments do illustrate is that the unit of selection can be influenced by a complex set of interactions between selection operating upon individuals, mode of reproduction, physical linkage and the spectrum of realized genetic diversity. The unit of selection problem therefore lies at the center of much of modern population genetics.

We are very grateful to DR. HAMPTON L. CARSON for providing encouragement and guidance as well as the stocks of *Drosophila mercatorum* and to DR. GEORGE BREWER for the use of some of his electrophoretic equipment. We appreciate the constructive criticisms of an earlier draft made by DR. CHRISTOPHER WILLS and an anonymous reviewer. We thank the society of Fellows of the University of Michigan for the opportunity afforded by a Junior Fellowship to TEMPLETON.

LITERATURE CITED

ASHER, J. H., JR., 1970 Parthenogenesis and genetic variability. II. One-locus models for various diploid populations. Genetics **66**: 369–391.

BREWER, G. J., 1970 *An Introduction to Isozyme Techniques.* Academic Press, N.Y.

BRNCIC, D., 1954 Heterosis and the integration of the genotype in geographical populations of *Drosophila pseudoobscura.* Genetics **39**: 77–88.

CARSON, H. L., 1967a Selection for parthenogenesis in *Drosophila mercatorum.* Genetics **55**: 157–171. ——, 1967b Permanent heterozygosity. Evol. Biol. **1**: 143–168. ——, 1973 The genetic system in parthenogenetic strains of *Drosophila mercatorum.* Proc. Natl. Acad. Sci. U.S. **70**: 1772–1774.

CARSON, H. L. and S. H. SNYDER, 1972 Screening by parthenogenesis for induced mutations in *Drosophila mercatorum.* Egypt. J. Gen. Cytol. **1**: 256–261.

CARSON, H. L., I. Y. WEI and J. A. NIEDERKORN, JR., 1969 Isogenicity in parthenogenetic strains of *Drosophila mercatorum.* Genetics **63**: 619–628.

CROW, J. F., 1957 Genetics of DDT resistance in *Drosophila.* Proc. Internatl. Genetics Symposum, 1956: 408–409.

DE MAGALHAES, I. E., J. S. DE TOLEDO and A. B. DA CUNHA, 1965 The nature of lethals in *Drosophila willistoni.* Genetics **52**: 599–608.

DOBZHANSKY, TH., 1948 Genetics of natural populations XVIII. Experiments on chromosomes of *Drosophila pseudoobscura* from different geographical regions. Genetics **33**: 588–602. ——, 1950 Genetics of natural populations. XIX. Origin of heterosis through natural selection in populations of *Drosophila pseudoobscura.* Genetics **35**: 288–302.

DOBZHANSKY, TH., B. SPASSKY and W. ANDERSON, 1965 Bichromosomal synthetic semilethals in *Drosophila pseudoobscura.* Proc. Natl. Acad. Sci. U.S. **65**: 345–348.

FRANKLIN, I. and R. C. LEWONTIN, 1970 Is the gene the unit of selection? Genetics **65**: 707–734.

HILDRETH, P. E., 1956 The problem of synthetic lethals in *Drosophila melanogaster.* Genetics **41**: 729–742.

MAYR, E., 1970 *Populations, Species, and Evolution.* Harvard U. Press, Cambridge.

MCFARQUHAR, A. M. and F. W. ROBERTSON, 1963 The lack of evidence for co-adaptation in crosses between races of *Drosophila subobscura* Coll. Genet. Res. **4**: 104–131.

MOLL, R. H. and C. W. STUBER, 1971 Comparisons and response to alternative selection procedures initiated with two populations of maize (Zea mays, L.). Crop Sci. **11**: 706–711.

NACE, G. W., C. M. RICHARDS and J. H. ASHER, JR., 1970 Parthenogenesis and genetic variability. I. Linkage and inbreeding estimations in the frog, *Rana pipiens.* Genetics **66**: 349–368.

RICHARDSON, R. H. and K. KOJIMA, 1965 The kinds of genetic variability in relation to selection responses in Drosophila fecundity. Genetics **52**: 583–598.

SING, C. F. and A. R. TEMPLETON, 1975 A search for the genetic unit of selection. In: *Isozymes: Genetics and Evolution.* Edited by C. L. MARKERT. Academic Press, N.Y.

SINGH, B. N., 1972 The lack of evidence for coadaptation in geographic populations of *Drosophila ananassai.* Genetica **43**: 582–588.

SLATKIN, M., 1972 On treating the chromosome as the unit of selection. Genetics **72**: 157–168.

TEMPLETON, A. R. and E. D. ROTHMAN, 1973 The population genetics of parthenogenetic strains of *Drosophila mercatorum.* I. One locus model and statistics. Theoret. Appl. Genetics **43**: 204–212.

TEMPLETON, A. R., H. L. CARSON and C. F. SING, 1976 The population genetics of parthenogenetic strains of *Drosophila mercatorum*. II. The capacity for parthenogenesis in a natural, bisexual population. Genetics, in press.

WALLACE, B., 1968 *Topics in Population Genetics*. W. W. Norton & Co., N.Y.

WALLACE, B., J. C. KING, C. V. MADDER, B. KAUFMANN and E. C. MCGUNNIGLE, 1953 An analysis of variability arising through recombination. Genetics **38**: 272–307.

WILLS, C., J. CRENSHAW and J. VITALE, 1970 A computer model allowing maintenance of large amounts of genetic variability in Mendelian populations. I. Assumptions and results for large populations. Genetics **64**: 107–123.

Corresponding editor R. C. LEWONTIN