EXPERIMENTAL DETERMINATION OF FITNESS INTERACTIONS IN *DROSOPHILA MELANOGASTER* BY THE METHOD OF MARGINAL POPULATIONS

JEROME WILSON[1,2]

Department of Life Sciences, University of California, Riverside, California 93502
Department of Zoology, University of Chicago, Chicago, Illinois 60637

Received December 4, 1967

IN the last decade the theoretical consequences of the joint effects of recombination rate and fitness interactions between loci have been studied by KIMURA (1956, 1965), KOJIMA (1959 a,b), LEWONTIN and KOJIMA (1960), LEWONTIN (1964 a,b,c), and FELSENSTEIN (1965). Several observations support the relevance of this body of theory to natural and laboratory populations.

Non-random associations of inversions in natural populations may be the result of fitness interaction between the inversions (LEVITAN, 1958; LEVITAN and SALZANO 1959; STALKER 1960; WHITE, LEWONTIN and ANDREW 1963). Viability interactions between inversions have been demonstrated experimentally (SPASSKY, DOBZHANSKY and ANDERSON 1965) and in nature (LEWONTIN and WHITE 1960). CANNON (1963) reported an increase in linkage disequilibrium for markers introduced into laboratory populations of *Drosophila melanogaster*. This suggests, among several hypotheses, fitness interactions between the marked loci. The non-random association of shell color and banding pattern in *Cepaea nemoralis* (CAIN and SHEPPARD, 1954)—characters controlled primarily by two linked loci (CAIN, KING and SHEPPARD 1960)—indicates a fitness interaction underlaid by differential predation.

A survey for fitness interactions, which implies estimation of a genotypic fitness array of a population segregating for two or more loci, has not been conducted on successive generations of laboratory populations. This may be due to technical difficulties, *viz.* the geometrical increase of the number of different genotypic classes to be scored and fitnesses to be estimated with each additional segregating locus, the estimation of linkage disequilibrium, the sparse representation of some genotypic classes leading to unreliable estimates of their fitnesses, and the lack of independence between estimates. However, fitness estimation in the one-locus "marginal" populations described below yields information about the presence or absence of fitness interactions in multilocus populations. At the same time, the problem of estimation of linkage disequilibrium is removed and the other difficulties of multilocus fitness estimation are diminished.

There is a pitfall in fitness estimation with one-locus populations that is per-

[1] Supported by Public Health Service Predoctoral Fellowship 1-F1-GM-30, 01A1.
[2] Present address: Department of Zoology, University of Chicago, Chicago, Illinois 60637.

haps not widely appreciated, although discussed by PROUT in 1965. PROUT considered a population starting from newly formed zygotes on which selection acts in two stages. The first stage of selection acts upon the genotypic distributions of zygotes up to the moment of observation. The second stage of selection further modifies the genotypic distribution of the population to produce an effective end-of-generation genotypic distribution. These fully-selected genotypes then mate and produce the zygotes of the next generation which are again selected in two stages. The population is observed at the same point of the life cycle in each successive generation. PROUT assumed that, for each genotype, the "early" and "late" components of fitness of each genotype were constant over all generations. Therefore, the net fitness of a genotype (which is the product of the two components of fitness of that genotype) is constant over all generations.

Using the case of two alleles at one locus and random mating, he explored the consequences of estimation of fitnesses from the observed genotypic distributions of successive generations. He found that the fitness estimators are biased and that the fitness estimated from successive generations of a non-equilibrium population will give a spurious appearance of frequency-dependent selection (i.e., the fitness estimates of a genotype will tend to increase as the genotype becomes rarer). PROUT excluded from this conclusion the two unlikely cases that (1) there is no second stage selection and that (2) the selection pattern is formally equivalent to gametic selection in both stages of selection.

Fitness estimation from the genotypic distributions of successive generations of *Drosophila melanogaster* reported below takes into account both the pre-observation and post-observation components of fitness for each genotype. Estimation of the two fitness components separately yields, as well as estimates of net fitness, a comparison of the pre- and post-observation components of each genotype. The comparisons are used to examine the generality of positive correlation of fitness components.

MATERIALS AND METHODS

Fitness interactions in marginal populations: "Fitness interaction" here means a deviation from the multiplicative (or log-additive) model. Figure 1 gives an example of a multiplicative fitness array for a two-locus population with two alleles at each locus. The figure also shows how a two-locus population is partitioned into one-locus "marginal" populations.

The same array, excluding the double heterozygote, characterizes four one-locus "marginal" populations. They are the *A* locus segregating on (1) homozygous *B/B* and on (2) homozygous *b/b* backgrounds, and the *B* locus segregating on (3) homozygous *A/A* and on (4) homozygous *a/a* backgrounds. Opposite marginal populations segregate for one locus on two homozygous alternatives at the other locus. (e.g. Populations 1 and 2 are opposite marginal populations because they both segregate for the *A* locus on the homozygous alternatives at the *B* locus, *B/B* and *b/b*.)

If a two-locus array conforms to the multiplicative model, opposite marginal populations have proportional fitness arrays and are expected to exhibit identical gene frequency kinetics. If a corresponding genotype is chosen from each population as a standard of fitness equal to 1 (e.g. the *A/a* heterozygote from populations 1 and 2), the corresponding genotypic fitnesses within each population are equal, relative to the fitness standard. Therefore, estimates of fitness of a given *A* locus genotype should not differ significantly from one homozygous state to the

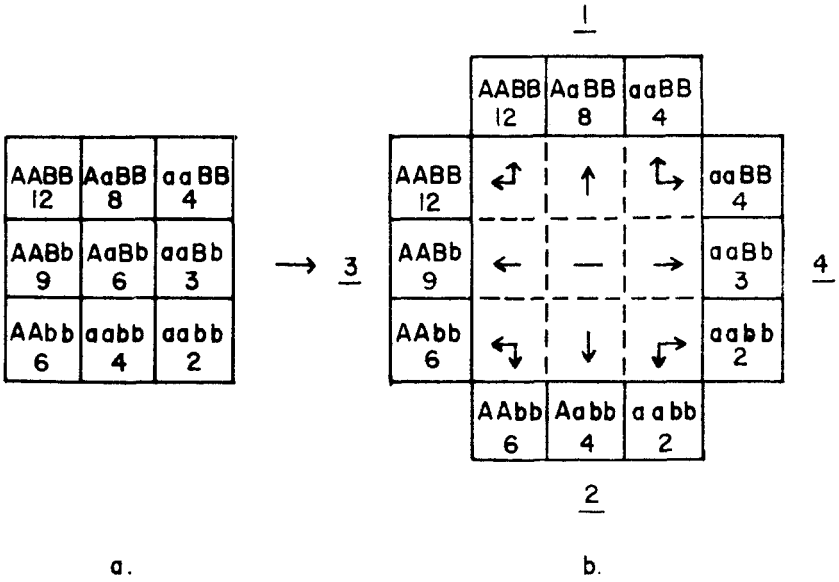

FIGURE 1.—a. Two-locus genotypic array with multiplicative fitnesses. b. Subdivision of two-locus population into one-locus marginal populations.

other at the *B* locus. Significant difference of the average fitness estimates of corresponding genotypes in opposite marginal populations indicates a fitness interaction between the loci.

Format of experimental populations: All populations were maintained on corn meal medium in half-pint milk bottles. The populations were established with fifty pairs of flies which were removed after seven days. At fourteen days all flies were classified according to genotype, the males discarded and the inseminated females placed in fresh bottles. Thereafter, the cycle of seven days of egg-laying, discarding the females, clearing and counting at fourteen days, discarding the males and placing the females into fresh bottles was continued until the populations were terminated. The bottles were held at 25°C except during counting. This regimen produced discrete generation populations with high egg and larval density.

Markers and populations: The X-chromosome markers used were *Bar* (*B*: 1–57.0) and *Beadex* (*Bx*: 1–58.4) and their wild-type alleles ($+^{B}$ and $+^{Bx}$). The two one-locus populations formed from these markers were the *Bar*-locus segregating on (1) homozygous *Bx/Bx* and on (2) homozygous $+^{Bx}/+^{Bx}$. These populations are opposite marginal populations of a two-locus population segregating for the *Bar* and *Beadex* loci.

The autosomal markers used were at the *spineless* locus (*spineless* (*ss*: 3–58.5) and *aristapedia* (ss^{a})), the *Dichaete* locus ((*D*: 40.4 to 41.0) and its allele $+^{D}$), and the *Lyra* locus ((*Ly*: 3–40.5) and its allele $+^{Ly}$). *Dichaete* and *Lyra* are homozygous lethal. These markers are used to form populations in which the *Dichaete* locus segregated on homozygous *ss/ss* and on homozygous ss^{a}/ss^{a}, and in which the *Lyra* locus segregated on homozygous *ss/ss* and on homozygous ss^{a}/ss^{a}. These populations represent, respectively, the opposite margins of one two-locus population segregating at the *spineless* and *Dichaete* loci and of another segregating at the *spineless* and *Lyra* loci. In the populations described above, each genotype is phenotypically distinguishable.

All of the stocks used to start the populations segregating for *Bar* were derived from a single-pair mating of a *Bar* male and *Beadex* female. Each X-chromosome in the stocks was derived by recombination between the *Bar* and *Beadex* loci. All autosomal stocks were derived from a single-pair mating of ss/ss^{a} male and *D/Ly* female and each third chromosome in the stocks was derived from recombination between *D/Ly* and ss/ss^{a}.

Fitness estimation model: The model used to estimate fitnesses assumed that selection occurs in two stages in each generation. The first stage acts on the zygotic distribution to produce the partially selected distribution of adults, which are observed and classified. The second stage of selection further modifies the previously observed, partially selected genotypic distribution to produce a fully selected adult genotypic distribution. The fully selected adults then mate at random to produce the zygotes of the next generation.

In the experimental regimen described above, the first stage of selection is relative viability from zygote to the time of genotypic classification. For males, the second stage of selection is relative ability to mate, and fertility when they do mate. For females, the second stage of selection includes, as well as fertility and mating ability, relative fecundity and viability after transfer to the bottle of fresh medium. The component of fitness selected in the second stage is called the "mating component" in both sexes.

Fitness estimation procedure: Figure 2 indicates two successive generations (one generation interval). For illustrative purposes, differences of genotypic frequency and fitnesses between the sexes are ignored. The figure shows that, given the observed genotypic distribution of the first generation and the estimates of the viability and mating components of the genotypic fitnesses, the expected genotypic array of partially selected adults in the second generation can be readily computed. At the same time there is the observed genotypic distribution of partially selected adults in the second generation with which to compare the computed expectations. The goodness of fit of the observations to the expectations is then measured by the probability associated with the chi square computed from these observations and expectations and the appropriate degrees of freedom. By extension, the goodness of fit over several generation intervals is measured by the probability associated with the total chi square and degrees of freedom over these generation intervals.

Assuming that the viability and mating components of fitness of each genotype are constant over all generation intervals but unknown, the same procedure is used to estimate these components of fitness. That is, an arbitrary set of values for the fitness components can be assumed and the total chi square computed. Among all the sets of fitness components, the set which minimizes the total square is taken as the best set of estimates. The number of degrees of freedom available to test the model is (number of generation intervals $\times$ number of independent genotype classes)—(number of parameters estimated). For instance, taking three generation intervals of the population described in Figure 2 and estimating all four fitness components, the total chi square will be distributed with two degrees of freedom if the model is correct.

Although in principle all the components of fitness can be estimated by this procedure, the viability components were estimated independently and taken as known, the minimum chi square procedure being used to estimate the mating components of fitness. The viabilities and their standard errors were estimated by maximum likelihood from F_1 and backcross data. The viability experiments were similar to the first two generations of the experimental populations. Fifty males and fifty newly emerged virgin females were allowed to mate for three days in each replicate bottle, etherized, the males discarded, the females placed in fresh bottles for seven days, and then discarded. The progeny were collected and tabulated fourteen days after the inseminated parental females were placed in the bottles. Comparisons of viability were made in both backcross and heterozygote $\times$ heterozygote matings. The sizes of the progeny samples were approximately 2000 for the heterozygote matings and 1000 for each kind of backcross mating.

RESULTS

The viability estimates of the various genotypes are listed in Table 1. Those genotypes with viability of 1.00 are the standards with which the other genotypes are compared.

These viability estimates were used in turn to obtain the mating component estimates. The same genotypes were taken as standards for the mating component ($= 1.00$) as for the standards of viability. Thus, the net fitness of the standard

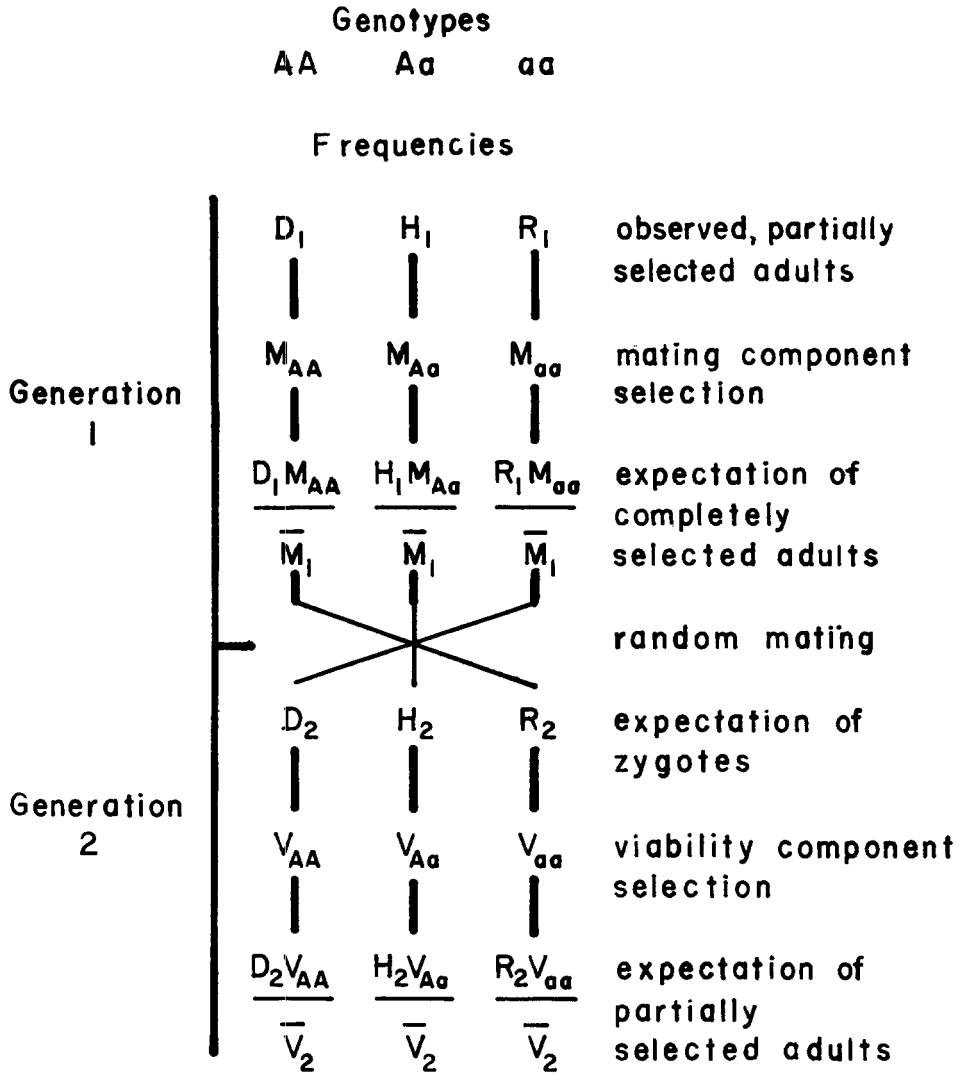

FIGURE 2.—Computation of expected genotype array at observation point from observation of previous generation. Selection divided into viability and mating components.

genotype was 1.00. The following tables present the estimates of net fitness (viability estimate × mating component estimate) of each genotype relative to the appropriate standard. The set of fitnesses was estimated in each of several replicate populations. This gives an empirical mean and distribution for the fitness estimates of each genotype. The tables provide a "t" test of significance of the difference of the mean fitness estimates for genotypes alike at one locus but differing in the homozygous state at the other locus. A significant difference between the mean fitness estimates of corresponding genotypes (P less than 5%) indicates a fitness interaction between the two loci. Because the fitnesses are esti-

TABLE 1

Viability estimates ± 2× standard error

		ss/ss ♂	ss/ss ♀	ss^a/ss^a ♂	ss^a/ss^a ♀
	$+^D/+^D$	1.00	1.00	1.00	1.00
	$+^D/D$	.75 ± .08	.78 ± .08	.85 ± .10	.85 ± .10
	$+^{Ly}/+^{Ly}$	1.00	1.00	1.00	1.00
	$+^{Ly}/L\gamma$	.77 ± .10	.87 ± .09	.79 ± .10	.68 ± .09

	$+^{Bx}$ ♂		$+^{Bx}/+^{Bx}$ ♀		Bx ♂		Bx/Bx ♀
$+^B$	1.00	$+^B/+^B$	1.05 ± .09	$+^B$	1.00	$+^B/+^B$	1.03 ± .11
B	.85 ± .07	$+^B/B$	1.00	B	.81 ± .12	$+^B/B$	1.00
		B/B	.77 ± .09			B/B	.88 ± .14

mated relative to a standard within each kind of population, it is emphasized that the comparison of average fitness estimates across two kinds of populations yields only information about fitness interactions: no inference is made about the relative competitive ability of the two genotypes if they were to be compared in the same population. The tables also include the total chi square and degrees of freedom for each replicated population. The chi squares are usually not significant. Therefore, the model is adequate.

Table 2 shows two significant differences between average fitness estimates

TABLE 2

Fitness estimates of Bar *locus segregants*

Fitness estimates				Fitness estimates			
B♂ Bx	$+^B/+^B$♀ Bx/Bx	B/B♀ Bx/Bx	χ^2/df	B♂ $+^{Bx}$	$+^B/+^B$♀ $+^{Bx}/+^{Bx}$	B/B♀ $+^{Bx}/+^{Bx}$	χ^2/df
.40	1.01	.22	6.5/12	.18	.80	.26	16.8/12
.23	.77	.15	8.5/12	.13	.89	.63	7.2/12
.18	.79	.20	9.4/9	.16	.76	.42	9.0/15
.28	.91	.36	10.0/12	.08	1.01	.38	13.3/12
.23	1.46	.17	2.3/6	.14	.95	.90	12.9/9
.20	1.18	.23	4.1/6	.08	.95	.84	2.9/9
.15	1.27	.14	1.7/6	.14	.94	.38	14.1/9
			Means				
.23	1.04	.22		.13	.90	.54	

't' test of average estimates

	B♂ Bx , B♂ $+^{Bx}$	$+^B/+^B$♀ Bx/Bx , $+^B/+^B$♀ $+^{Bx}/+^{Bx}$	B/B♀ Bx/Bx , B/B♀ $+^{Bx}/+^{Bx}$
t_{14}	3.1	1.5	3.9
P	< .01	> .10	< .01

't' test of significant difference between average fitness estimates of corresponding *Bar* locus genotypes for alternative states at the *Beadex* locus. Genotypic fitness standards are $+^B$♂ and $+^B/B$♀.

and, hence, a fitness interaction between the *Bar* and *Beadex* loci. The *Bar* males have higher average fitness when hemizygous *Beadex* than when hemizygous $+^{Bx}$. The homozygous *Bar* females have higher average fitness when homozygous $^{Bx}+$ than when homozygous *Bx*. The difference of the average fitness estimates between homozygous $+^{B}$ females is not significant.

There is a fitness interaction between the *spineless* locus and the *Dichaete* locus (Table 3). *Dichaete* males (heterozygotes) have higher average fitness when homozygous *aristapedia* than when homozygous *spineless*. The heterozygous *Dichaete* females do not differ significantly across homozygous states at the *spineless* locus.

The *Lyra* and *spineless* loci interact also, heterozygous *Lyra* females having significantly higher average fitness when homozygous *spineless* than when homozygous *aristapedia* (Table 4). The heterozygous *Lyra* males do not differ significantly with respect to the homozygous state at the spineless locus.

DISCUSSION

In each two-locus comparison, a fitness interaction in one or both sexes was encountered. It is not possible to extrapolate from these results to the effect of these interactions on the gene frequency kinetics of the appropriate two-locus population.

A comparison of gene frequency kinetics between populations segregating at one locus and homozygous for alternative alleles at the other locus does not always reveal an existing fitness interaction. Part of the reason for this is simply because the gene frequency statistic confounds so much information. When the popula-

TABLE 3

Fitness estimates of Dichaete *locus segregants*

Fitness estimates			Fitness estimates		
$+^{D}/D\,♂$ ss/ss	$+^{D}/D\,♀$ ss/ss	χ^2/df	$+^{D}/D\,♂$ ss^a/ss^a	$+^{D}/D\,♀$ ss^a/ss^a	χ^2/df
.57	.78	14.3/18	.65	.85	10.3/14
.58	.39	10.6/6	.78	.61	20.5/10
.37	.42	14.4/12	.78	.61	3.4/4
.51	.32	3.6/4	.80	.35	10.4/10
Means					
.51	.51		.75	.60	
't' test of average estimates					
		$+^{D}/D\,♂$ ss/ss , $+^{D}/D\,♂$ ss^a/ss^a		$+^{D}/D\,♀$ ss/ss , $+^{D}/D\,♀$ ss^a/ss^a	
	t_6	4.03		0.69	
	P	$< .01$		$> .50$	

't' test of significant difference between average fitness estimates of corresponding *Dichaete* locus genotypes for alternative homozygous states at the *spineless* locus. Genotypic fitness standards are $+^{D}/+^{D}$ ♂ and ♀.

TABLE 4

Fitness estimates of Lyra *locus segregants*

Fitness estimates			Fitness estimates		
$+^{Ly}/Ly$♂ ss/ss	$+^{Ly}/Ly$♀ ss/ss	χ^2/df	$+^{Ly}/Ly$♂ ss^a/ss^a	$+^{Ly}/Ly$♀ ss^a/ss^a	χ^2/df
.12	.59	.8/4	.11	.46	2.2/4
.08	.51	.5/4	.54	.39	8.7/4
.28	.47	4.8/6	.54	.10	2.1/4
.35	.49	1.6/4	.33	.18	2.2/4
		Means			
.21	.52		.38	.28	
		't' test of average estimates			
		$+^{Ly}/Ly$♂ ss/ss , $+^{Ly}/Ly$♂ ss^a/ss^a		$+^{Ly}/Ly$♀ ss/ss , $+^{Ly}/Ly$♀ ss^a/ss^a	
	t_6	1.41		2.65	
	P	$> .20$		$< .05$	

't' test of significant difference between average fitness estimates of corresponding *Lyra* locus genotypes for alternative homozygous states at the *spineless* locus. Genotypic fitness standards *are* $+^{Ly}/+^{Ly}$ ♂ and ♀.

tions being compared are segregating for a lethal, the differences in change of gene frequency attributable to difference in fitness of the heterozygotes would be swamped by the lethality of one of the homozygous classes unless the fitness interaction were prodigious. Figure 3 shows the changes of gene frequency plotted against gene frequency of the *Dichaete* and *Lyra* alleles in their respective populations. The effect of interaction between the *Dichaete* or *Lyra* locus and the *spineless* locus is not apparent in this mode of presentation. This is also true of the gene frequency kinetics of the segregating *Bar* locus (Figure 4). Here, however, the effect of fitness interaction is diminished by being "sex-cancelling". That is, the marginal fitness of the *Bar* allele falls in the males and rises in the females when the background changes from homozygous *Bx* to homozygous $+^{Bx}$.

The estimates of viability, mating ability, and net fitness of each genotype are

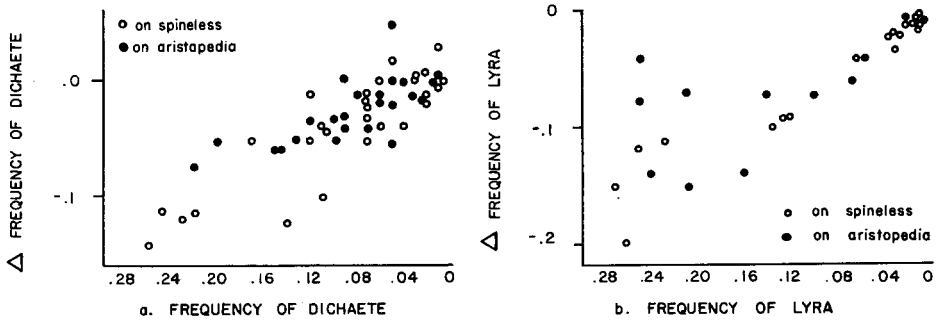

FIGURE 3.—a. *Dichaete* locus segregating on homozygous *spineless* locus backgrounds. b. *Lyra* locus segregating on homozygous *spineless* locus backgrounds.

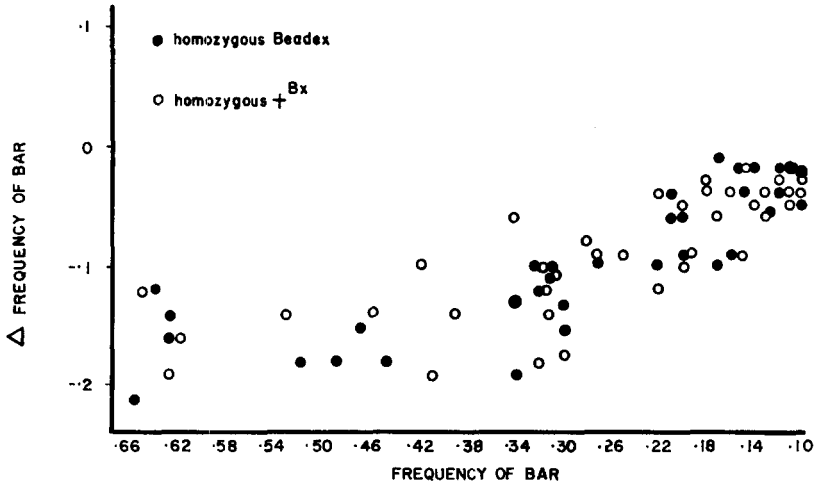

FIGURE 4.—*Bar* locus segregating on homozygous *Beadex* locus backgrounds.

given in Table 5. The correlation coefficient for viability and the mating component is 0.45 which is not significantly different from zero at the 5% level ($0.10 > P > 0.05$). On the face of it, these data fail to support the hypothesis of correlation between the fitness components. However, the method of estimation tends to underestimate one component when it overestimates the other. The relative importance of this consideration is difficult to evaluate when minimum chi square estimation is utilized. If the estimate of correlation were significantly positive, it is still too low to justify the prediction of net fitness from viability alone.

TABLE 5

Viability component, mating component, and net fitness of genotypes relative to a standard genotype. r = estimate of correlation between mating and viability components

Genotype	Standard	Viability	Mating (Mean estimate)	Net fitness (Mean estimate)
$B\ Bx/Y\ ♂$	$+^{B}\ Bx/Y\ ♂$	0.81	0.28	0.23
$+^{B}\ Bx/+^{B}\ Bx\ ♀$	$+^{B}\ Bx/B\ Bx\ ♀$	1.03	1.01	1.04
$B\ Bx/B\ Bx\ ♀$	$+^{B}\ Bx/B\ Bx\ ♀$	0.88	0.25	0.22
$B\ +^{Bx}/Y\ ♂$	$+^{B}\ +^{Bx}/Y\ ♂$	0.85	0.15	0.13
$+^{B}\ +^{Bx}/+^{B}\ +^{Bx}\ ♀$	$+^{B}\ +^{Bx}/B\ +^{Bx}\ ♀$	1.05	0.86	0.90
$B\ +^{Bx}/B\ +^{Bx}\ ♀$	$+^{B}\ +^{Bx}/B\ +^{Bx}\ ♀$	0.74	0.70	0.54
$+^{Ly}\ ss/Ly\ ss\ ♂$	$+^{Ly}\ ss/+^{Ly}\ ss\ ♂$	0.79	0.27	0.21
$+^{Ly}\ ss/Ly\ ss\ ♀$	$+^{Ly}\ ss/+^{Ly}\ ss\ ♀$	0.87	0.59	0.52
$+^{Ly}\ ss^{a}/Ly\ ss^{a}\ ♂$	$+^{Ly}\ ss^{a}/+^{Ly}\ ss^{a}\ ♂$	0.79	0.48	0.38
$+^{Ly}\ ss^{a}/Ly\ ss^{a}\ ♀$	$+^{Ly}\ ss^{a}/+^{Ly}\ ss^{a}\ ♀$	0.68	0.41	0.28
$+^{D}\ ss/D\ ss\ ♂$	$+^{D}\ ss/+^{D}\ ss\ ♂$	0.75	0.68	0.51
$+^{D}\ ss/D\ ss\ ♀$	$+^{D}\ ss/+^{D}\ ss\ ♀$	0.78	0.65	0.51
$+^{D}\ ss^{a}/D\ ss^{a}\ ♂$	$+^{D}\ ss^{a}/+^{D}\ ss^{a}\ ♂$	0.85	0.89	0.75
$+^{D}\ ss^{a}/D\ ss^{a}\ ♀$	$+^{D}\ ss^{a}/+^{D}\ ss^{a}\ ♀$	0.85	0.71	0.60

$\hat{r}_{M,V} = 0.45$: $\quad 0.10 > P_{r=0} > 0.05$

In twelve of the fourteen cases, the mating component of fitness is more critical to net fitness than the viability component, relative to the genotypic standards, i.e., the mating components are much more variable and differ by more from the standards than the viability components. Because the standards of fitness were chosen from among the more fit genotypes, the less fit genotypes suffer more from their inabilities directly connected to reproduction than from inviability.

Where the possibility exists of post-observation selection in natural or laboratory populations, at least three consequences arise in not estimating this component. In the case where the post-observation component is acknowledged but not estimated, conclusions about future gene frequency changes of the population based on viability alone must necessarily be tentative (as in LEWONTIN and WHITE 1960). When the post-observation component is not acknowledged, fitness estimation may lead to the spurious appearance of frequency-dependent selection (possibly the case in POLIVANOV 1964, TOBARI and KOJIMA 1967, and YARBROUGH and KOJIMA 1967). Finally, it may be impossible to obtain a good fit between model and observation when the model does not take into account the post-observation component of selection. This last may be the difficulty encountered by LEVENE, PAVLOVSKY and DOBZHANSKY 1954) in estimating the fitness of morphs in a polymorphism involving three allelic gene arrangements.

SUMMARY

Fitness interactions between two loci may be assayed by examining the fitnesses of genotypes in each of two one-locus populations, which segregate for the same alleles at one locus but differ in the homozygous state at the second locus. Fitness estimation in appropriate one-locus, discrete generation populations of *D. melanogaster* revealed a fitness interaction in one or both sexes for each of the three pairs of loci tested. The fitness interactions did not imply a dramatic difference in the gene frequency kinetics of the segregating locus between two homozygous states at the same locus, however. The model of fitness estimation assumed random mating and two components of fitness for each genotype. One component was viability from egg to the time of observation of the adult. The other component compounded the capabilities of the genotype directly connected to reproduction (the mating component).

LITERATURE CITED

CANNON, G. B., 1963 The effect of natural selection on linkage disequilibrium and relative fitness in experimental populations of *Drosophila melanogaster*. Genetics **48**: 1201–1216.

CAIN, A. J., and P. M. SHEPPARD, 1954 Natural selection in Cepaea. Genetics **39**: 89–116.

CAIN, A. J., J. M. B. KING, and P. M. SHEPPARD, 1960 New data on the genetics of polymorphism in the snail *Cepaea nemoralis* L. Genetics **45**: 393–411.

FELSENSTEIN, P., 1965 The effect of linkage on directional selection. Genetics **52**: 349–363.

KIMURA, M., 1956 A model of a genetic system which leads to closer linkage by natural selection. Evolution **10**: 278–287. —— 1965 Attainment of quasi-linkage equilibrium when gene frequencies are changing by natural selection. Genetics **52**: 875–890.

KOJIMA, K., 1959a Role of epistasis and overdominance in stability of equilibria with selection. Proc. Natl. Acad. Sci. U.S. **45**: 984–989. —— 1959b Stable equilibria for the optimum model. Proc. Natl. Acad. Sci. U.S. **45**: 989–993.

LEVENE, H., O. PAVLOVSKY, and TH. DOBZHANSKY, 1954 Interaction of adaptive values in polymorphic experimental populations of *Drosophila pseudoobscura*. Evolution **8**: 335–349.

LEVITAN, M., 1958 Non-random association of inversions. Cold Spring Harbor Symp. Quant. Biol. **23**: 251–268.

LEVITAN, M., and F. M. SALZANO, 1959 Studies of linked inversions in *Drosophila guaramunu*. Heredity **13**: 243–248.

LEWONTIN, R. C., 1964a The role of linkage in natural selection. Proc. 11th Intern. Congr. Genetic. **3**: 517–525. —— 1964b The interaction of selection and linkage. I. General considerations; heterotic models. Genetics **49**: 49–67. —— 1964c The interaction of selection and linkage. II. Optimum models. Genetics **50**: 757–782.

LEWONTIN, R. C., and K. KOJIMA, 1960 The evolutionary dynamics of complex polymorphisms. Evolution **14**: 458–472.

LEWONTIN, R. C., and M. J. D. WHITE, 1960 Interaction between inversion polymorphisms of two chromosome pairs in the grasshopper *Moraba scurra*. Evolution **14**: 116–129.

POLIVANOV, S., 1964 Selection in experimental populations of *Drosophila melanogaster* with different genetic backgrounds. Genetics **50**: 81–100.

PROUT, T., 1965 The estimation of fitness from genotypic frequencies. Evolution **19**: 546–551.

SPASSKY, B., TH. DOBZHANSKY, and W. W. ANDERSON, 1965 Genetics of natural populations. XXXVI. Epistatic interactions of the components of genetic load in *Drosophila pseudoobscura*. Genetics **52**: 653–664.

STALKER, H. D., 1960 Chromosomal polymorphism in *Drosophila paramelanica* Patterson. Genetics **45**: 95–114.

TOBARI, Y. N., and K. KOJIMA, 1967 Selective modes associated with inversion karyotypes in *Drosophila ananassae*. I. Frequency-dependent selection. Genetics **57**: 179–188.

WHITE, M. J. D., R. C. LEWONTIN, and L. E. ANDREW, 1963 Cytogenetics of the grasshopper *Moraba scurra*. VII. Geographic variation of the adaptive properties of inversions. Evolution **17**: 147–162.

YARBROUGH, K., and K. KOJIMA, 1967 The mode of selection at the polymorphic esterase 6 locus in cage populations of *Drosophila melanogaster*. Genetics **57**: 677–686.