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FACTORS AFFECTING THE GENETIC LOAD IN *DROSOPHILA*: SYNERGISTIC EPISTASIS AND CORRELATIONS AMONG FITNESS COMPONENTS

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Abstract.—Two factors that can affect genetic load, synergistic epistasis and sexual selection, were investigated in *Drosophila melanogaster*. A set of five chromosomal regions containing visible recessive mutations were put together in all combinations to create a full set of 32 homozygous lines fixed for different numbers of known mutations. Two measures of fitness were made for each line: productivity (a combined measure of fecundity and egg-to-adult survivorship) and competitive male mating success. Productivity, but not male mating success, showed a pattern of strong average synergistic epistasis, such that the log fitness declined nonlinearly with increasing numbers of mutations. Synergistic epistasis is known to reduce the mutation load. Both fitness components show some positive and some negative interactions between specific sets of mutations. Furthermore, alleles with deleterious effects on productivity tend to also diminish male mating success. Given that male mating success can affect relative fitness without changing the mean productivity of a population, these additional effects would lead to lower frequencies and lower fixation rates of deleterious alleles without higher costs to the mean fitness of the population.

Key words.—*Drosophila melanogaster*, genetic load, pleiotropy, synergistic epistasis.

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The concept of genetic load has had a long history in evolutionary biology, dating back to Haldane's discussions of the fitness consequences of recurrent deleterious mutations and the costs of substituting one allele for a more favorable allele. Today the deleterious effects of mutations are known to be important to a variety of interesting evolutionary processes, including the evolution of sex and recombination (Kondrashov 1982, 1988, 1994, 1995; Barton 1995; Otto and Feldman 1997; Barton and Charlesworth 1998), the evolution of ploidy (Kondrashov and Crow 1991; Perrot et al. 1991, Otto and Goldstein 1992, Jenkins and Kirkpatrick 1995), the evolution of selfing (Charlesworth 1998), and the evolutionary and ecological fate of small populations (Lande 1994; Schultz and Lynch 1997).

Mutations that affect fitness may arise at a rate as high as one per individual per generation (Mukai 1964; Mukai et al. 1972; Johnston and Schoen 1995; see review by Lynch et al. 1999). Many of these new alleles are deleterious and reduce the fitness of their carriers. It has been estimated that the average effect of a new mutation on fitness could be as large as -2% (Simmons and Crow 1977); if mutations were unchecked by selection, then the mean fitness of a population would rapidly decline.

In large populations, there is a balance reached between the effects of mutation bringing new deleterious alleles into a population and selection acting to reduce the frequency of those alleles. The frequency of deleterious mutations is therefore small, but greater than zero. The reduction in the mean fitness of a population caused by this low frequency of deleterious alleles is referred to as "mutation load." In small populations, selection is not always effective at keeping allele frequencies low, and sometimes deleterious alleles reach high frequency as a result of random genetic drift, causing the fitness of the population to decline. This "drift load" is

thought to be a danger for the long-term persistence of small populations (Land 1994; Lynch et al. 1995a,b).

The extent to which deleterious alleles reduce the *mean* fitness of a population depends upon which fitness components are affected by the alleles and in what way. It has long been argued that density-dependent processes can compensate for some genetic load and that the overall reduction in a population's fitness due to the number of deleterious mutations it carries is ameliorated by the reproductive excess of most populations (Wallace 1991). Furthermore, it seems clear that the effects of mutant alleles on the survivorship or female fecundity of a typical population may have effects on the capacity of a population to sustain itself, but in most populations the competitive mating ability of males may be reduced without changing the overall number of offspring produced by the population.

Therefore a strong factor that may contribute substantially to the amelioration of the genetic load of a population is what we might call the "good-genes" hypothesis. Good genes models postulate that female choice evolves because females gain an indirect benefit by mating with genetically superior males (e.g., Williams 1966). If males that carry more deleterious alleles are less likely to find mates than males with fewer harmful mutations, then the effect of sexual selection is to reduce the frequency of deleterious mutations without reducing the productivity of the population. As a result, the effective strength of selection against deleterious alleles is stronger than the contribution of these alleles to the load. Hughes (1995) found some evidence for a positive genetic correlation between male longevity and mating ability (see also Houle et al. 1994), and there is some evidence for such a correlation between mating success and offspring quality or quantity in the standing genetic variation as predicted by the good-genes models of sexual selection (e.g. Partridge 1980; Welch et al. 1998; see Andersson 1994). There is little information, however, about how the deleterious effects of new mutations on female fecundity are accompanied by de-

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clines in male mating success. Such a relationship could reduce the expected genetic load substantially (Whitlock 2000).

Genetic interactions among mutations may also contribute significantly to the amelioration of the genetic load of a population. If deleterious alleles interact synergistically (i.e., adding more deleterious alleles makes fitness decrease at an increasing rate), then the mutation load in sexual populations is expected to be much less than with independently acting mutations (Kimura 1961; Kimura and Maruyama 1966; Kondrashov 1982; Rice 1998). This is because the individuals that die remove on average more than one deleterious allele from the population. This does not happen in asexual populations, where mutations are eliminated independently following Muller's principle of "one mutation, one mutation death" (Muller 1950). Thus, the suggestion has been made that a major advantage of sex is that it provides a population with a lower mutation load (Crow 1970; Kondrashov 1982, 1988). In addition epistasis is known to play a major role in numerous evolutionary processes (for a review, see Phillips et al. 2000).

As such, the degree of synergistic epistasis among deleterious alleles is a subject of substantial interest in evolutionary biology, yet there is insufficient data to generalize about the extent of this epistasis. For years, the only direct information came from Mukai's (1969) study of the epistatic effects of mutations accumulated in lines of *Drosophila melanogaster*. Mukai's study found evidence for synergistic epistasis based on a quadratic relationship between log viability and the presumed number of accumulated mutations. This study is based on a number of assumptions, including that the period of mutation accumulation was proportional to the number of mutations accumulated, and the significance of the result rests on two datapoints that are not independent (Lynch and Walsh 1998).

Recently, many authors have looked for direct or indirect evidence of synergistic epistasis. Willis (1993) examined the relationship between the inbreeding coefficient and the degree of inbreeding depression and found little overall evidence for synergistic epistasis. This approach, as Willis suggests, is subject to the bias of selection during the inbreeding process itself, leading to an experimental design biased against finding synergism. Other indirect methods have included de Visser et al.'s (1997a) comparison of the skew of log fitness of a cross between distinct lines. Such a comparison has been applied to fitness-related traits in plants and fungi by de Visser and Hoekstra (1998). Their analysis suggested the presence of synergism for all of the traits studied in plants and for some in fungi, although the method is likely not extremely powerful (West et al. 1998).

Two recent direct tests of the degree of synergistic epistasis for fitness components have found little evidence for synergism on average, although both tests have found substantial variation in the type of interactions among different loci. Elena and Lenski (1997) generated 225 genotypes of *Escherichia coli* with varying numbers of mutations and measured their fitness. They found no evidence for synergistic epistasis on average, but instead found that some mutation combinations displayed negative epistasis while others displayed diminishing returns epistasis. Similarly, de Visser et al. (1997b) constructed lines with different combinations of

known deleterious alleles in *Aspergillus niger* and found no average epistasis. In this case, not all combinations of these alleles were generated, so that the worst combinations may have been removed by selection previous to testing. As a result, these data from *A. niger* cannot exclude synergistic epistasis.

We conducted an experiment to test for synergistic epistasis and the correlation between life-history components among a set of deleterious visible mutations in *D. melanogaster*. Five sets of visible recessive mutations and their wild-type alternatives were isolated into homozygous lines in all combinations. These lines were assessed for their productivity and the mating success of their males relative to a standard genotype. The results suggest the presence of some synergistic epistasis and that alleles that reduce productivity also decrease male mating success, two factors known to reduce genetic load.

MATERIALS AND METHODS

Derivation of Mutant Lines

Five mutant alleles (or pairs of alleles) were chosen for study in *D. melanogaster*. Each of these mutations is recessive and causes a visible phenotypic effect on the adult fly. These mutations (and mutation pairs) were *black* (*b*) and *plexus* (*px*)–*speck* (*sp*) on the second chromosome and *claret* (*ca*), *hairy* (*h*), and *ebony* (*e*)–*stripe* (*sr*) on the third chromosome. The pairs *px/sp* and *e/sr* are tightly linked and thus mark the same chromosomal regions. Descriptions of the effect of these alleles are given in Lindsley and Zimm (1992); they affect the body color, bristle patterns, wing venation patterns, and eye color of adult flies, with other pleiotropic effects.

The mutant alleles were taken from two chromosomes used for mapping, *all* and *rucuca*, obtained from the Bloomington Stock Center. The first strain, *all*, has seven recessive visible mutations on the second chromosome: *al*¹, *dp*^{ov1}, *b*¹, *pr*¹, *c*¹, *px*¹, and *sp*¹. The other strain, *rucuca*, has eight recessive visible mutations: *ru*¹, *h*¹, *th*¹, *st*¹, *cu*¹, *sr*¹, *e*^s, and *ca*¹. For each of these mapping chromosome lines, a stock was derived and made homozygous for both autosomes by two generations of crossing to a doubly balanced line, *CyO/Bl; Tm3/Gl*. *CyO* and *Tm3* are balancer chromosomes, which are marked with dominant alleles and do not allow recombination with wild-type chromosomes. By these crosses, two lines were created: one that was homozygous for a single *all* chromosome and a single wild-type third chromosome and another that was homozygous for a single wild-type second chromosome and a single *rucuca* chromosome.

These two homozygous strains were then crossed, and the resulting line was carried until the F₃, F₆, F₈, and F₁₀ generations, to allow for recombination between the marked and unmarked chromosomes. At that point, several lines were begun from a male recombinant fly crossed to *CyO/Bl; Tm3/Gl* females, then a male offspring from this cross was crossed again to *CyO/Bl; Tm3/Gl*. From the offspring of this cross, flies with the phenotype *CyO/+; Tm3/Gl* and *CyO/Bl; Tm3/+* were selected within each line and crossed with another fly of the same genotype from the same line, to create strains that were homozygous for one recombinant autosome and balanced at the other autosome: **/*; Tm3/Gl* or *CyO/Bl; */**,

where an asterisk represents a chromosome with some number of the visible mutations. At this point, the genotype of the unknown chromosome was revealed, and a line with each combination of alleles on each chromosome was established. Thus, at this stage there were four lines isolated for the second chromosome and eight for the third.

The four second-chromosome lines were then crossed to each of the eight third-chromosome lines to create all possible combinations of the marked regions. This was done by crossing individuals from a second-chromosome line to individuals from a third-chromosome line, selecting the doubly balanced offspring to cross, and then selecting among the F_2 for individuals without the balancer chromosomes and with the phenotypes of the marker alleles for that pair of chromosomes. All 32 combinations of these five marked chromosomal regions were obtained, thereby eliminating a potential source of strong bias in the measurement of epistasis for fitness.

These 32 mutant lines were maintained in vials for approximately eight generations before the fitness tests described below were conducted. All flies in this study were raised in standard food vials (75 × 25 mm diameter) with 7 ml of medium, kept at $25 \pm 1^\circ\text{C}$ with a constant illumination cycle of 12 h light followed by 12 h dark. All handling of the flies was performed at room temperature using CO_2 anesthesia.

Productivity Tests

The productivity of each of these 32 mutation lines was measured as a combined estimate of the fecundity of mutant females and the egg-to-adult survivorship of their offspring, in a standard competitive environment. The effects of rearing density on the fecundity of the females were controlled by raising flies from each line under constant density conditions. A large number of flies from each line were placed into larval collection cups (made from inverted urine specimen cups with normal fly media in the lid and a hole filled with a rayon ball for aeration). These flies were allowed to lay eggs for 4 h and then cleared. After 25 h, first-instar larvae were collected from the surface of the food in the collection cups and transferred 50 at a time to new vials. The flies that emerged from these vials were collected daily and transferred to new vials for holding, during which time the females were allowed to mate with males from their own lines for five days. This control for density was not perfect, however, because there were large differences in the survivorship of the vials. Some flies therefore experienced lower effective densities than would be expected from the starting numbers. Because it was the lines with more mutations that tended to have lower survivorship, this may bias the estimate of epistasis against finding synergistic epistasis, because the flies with higher numbers of mutations were able to grow in less crowded conditions.

Each replicate consisted of three mutant line females placed into a vial with three mated females from a marked strain of *D. melanogaster* with a visible mutation, *sparkling-poliert* (*spa^{pol}*). There were 24–95 replicates per line (mean number of replicates = 52.5). The females were allowed to lay eggs in the vials for 25 h, when they were removed. After

16 days, the adult offspring in these vials were counted by genotype.

Using the sparkling flies as a standard achieves several goals. First, they act as a standard to account for some of the variation among vials in quality. Second, the competitive environment of the mutant flies is determined largely by competition with a standard strain, and so the effects of differences in intragenotypic competitive effects are reduced.

Mating-Success Assays

We measured the mating success of males from each of the mutant lines by choice tests. For each replicate, one six- to eight-day-old male from one of the mutation lines was placed in a vial with a six- to eight-day-old male marked with *spa^{pol}* and a three- to six-day-old virgin female from the Dahomey stock population. These vials were checked for mating every 15 min for two hours. Mating pairs were removed, and the mating and nonmating males were scored under a dissecting microscope. Only those couples that remained in copula during movement to the microscope were scored as mating. The individual scoring the flies did not know the identity of the mutation line being tested. There were seven to 53 total matings per line (mean = 29.3).

Fitness Indices

For each measure of a fitness component, the numbers of offspring or matings by the experimental flies were summed over replicates and divided by the reproductive success of their paired *spa* flies. (For the analysis of variance, each productivity score was increased by one to prevent undefined logarithms.) The natural logarithm of this ratio is the measure of the log-relative fitness of that mutation line. This measure of log fitness would decline linearly with additional mutations if fitness were determined by a multiplicative interaction between alleles. The log-fitness score would decline with negative curvature if there were synergistic epistasis.

Statistical Analysis

The marked regions were treated as fixed effects. We are therefore testing hypotheses about the nature of the genetic relationships between these particular alleles. All parametric analyses were performed using JMP (SAS Institute 1995). A five-factor full factorial analysis of variance was performed looking for effects on productivity. The size of these effects was estimated using the Fit Model dialogue in JMP. Effects of the marker regions and interactions between them on male mating success were tested using logistic regression. Non-significant highest-order terms were sequentially dropped from the model, and effect sizes were estimated with the maximum-likelihood procedures in JMP.

RESULTS

Deleterious Effects of the Mutations

The fitness component estimates for each line are given in Table 1, and results from analysis of variance on productivity and logistic regression on male mating success are shown in Tables 2 and 3. Four of the five regions have deleterious

TABLE 1. The relative productivity and male mating success for each strain.

Strain	ln(relative productivity)	ln(male mating success)
+	-0.232	0.598
<i>px/sp</i>	-0.850	1.551
<i>b</i>	-0.312	0.182
<i>ca</i>	-0.214	0.118
<i>e/sr</i>	-0.847	-0.811
<i>h</i>	0.507	0.470
<i>px/sp/b</i>	-0.238	-0.234
<i>px/sp/ca</i>	-0.490	1.229
<i>px/sp/e/sr</i>	-1.030	-0.754
<i>px/sp/h</i>	0.232	0.154
<i>b/ca</i>	-0.968	-2.773
<i>b/e/sr</i>	-1.338	-2.793
<i>b/h</i>	-0.034	0
<i>ca/e/sr</i>	-1.47	-1.558
<i>ca/h</i>	-0.739	-0.811
<i>e/sr/h</i>	0.2176	-1.030
<i>px/sp/b/ca</i>	-0.712	-1.153
<i>px/sp/b/e/sr</i>	-1.820	-2.351
<i>px/sp/b/h</i>	-0.529	0.288
<i>px/sp/ca/e/sr</i>	-0.786	-1.504
<i>px/sp/ca/h</i>	-0.195	-0.932
<i>px/sp/e/sr/h</i>	-0.641	-0.916
<i>b/ca/e/sr</i>	-1.945	-1.012
<i>b/ca/h</i>	-0.047	*
<i>b/e/sr/h</i>	0.0264	-1.344
<i>ca/e/sr/h</i>	-1.296	-2.159
<i>px/sp/b/ca/e/sr</i>	-2.446	-2.079
<i>px/sp/b/ca/h</i>	-1.973	-1.163
<i>px/sp/b/e/sr/h</i>	-1.180	-2.303
<i>px/sp/ca/e/sr/h</i>	-1.024	-1.099
<i>b/ca/e/sr/h</i>	-1.856	*
<i>px/sp/b/ca/e/sr/h</i>	-4.560	-1.386

* These lines had no mating by mutant strain males, so the log ratio is undefined.

effects on log productivity, the exception being the region marked by *h*, which in this environmental context has a slight advantageous effect. Similarly, three of the mutations have a significant deleterious effect on male mating success: *b*, *ca*, and *e/sr*. The other two regions, with *h* and *px/sp*, have no significant main effects on mating success.

Epistatic Interactions for Fitness Components

The analysis of variance on log-relative productivity shows several significant interaction terms (Table 2). Six of the 10 two-way interactions are significant; in each of these six cases the direction of this effect is negative, that is, synergistic epistasis occurs. Five of the 10 three-way interactions are significant, and four of these are negative as well. Two of the four-way terms are significant, with one being a negative effect. Overall, there is a very strong pattern of negative epistasis for productivity in these data.

One alternative interpretation of these data, suggested by Jim Fry, is possible. The genetic region associated with the *h*¹ mutation has a significantly higher fitness than its wild-type counterpart (see Table 2). One might assume that the *h*¹ allele does not truly have a higher fitness than *h*⁺, but that the region marked by *h*⁺ carries some deleterious allele(s). If this were the case, then we would want to classify *h*⁺ as the deleterious allele. Doing this, each interaction term in-

TABLE 2. Results of analysis of variance for effects of alleles on log productivity.

Source	Estimate	P
<i>px/sp</i>	-0.217	< 0.0001
<i>b</i>	-0.375	< 0.0001
<i>ca</i>	-0.371	< 0.0001
<i>e/sr</i>	-0.437	< 0.0001
<i>h</i>	0.137	< 0.0001
<i>px/sp*b</i>	-0.128	0.0002
<i>px/sp*ca</i>	0.033	0.34
<i>px/sp*e/sr</i>	-0.017	0.62
<i>px/sp*h</i>	-0.193	< 0.0001
<i>b*ca</i>	-0.111	0.0011
<i>b*e/sr</i>	-0.132	0.0001
<i>b*h</i>	-0.048	0.16
<i>ca*e/sr</i>	-0.146	< 0.0001
<i>ca*h</i>	-0.207	< 0.0001
<i>e/sr*h</i>	0.031	0.36
<i>px/sp*b*ca</i>	-0.151	< 0.0001
<i>px/sp*b*e/sr</i>	-0.068	0.045
<i>px/sp*b*h</i>	-0.190	< 0.0001
<i>b*ca*e/sr</i>	-0.042	0.22
<i>b*ca*h</i>	0.032	0.35
<i>ca*e/sr*h</i>	-0.080	0.018
<i>px/sp*ca*e/sr</i>	0.044	0.20
<i>px/sp*ca*h</i>	0.006	0.87
<i>px/sp*e/sr*h</i>	-0.047	0.17
<i>b*e/sr*h</i>	0.078	0.022
<i>px/sp*b*ca*e/sr</i>	-0.004	0.91
<i>px/sp*ca*e/sr*h</i>	0.007	0.83
<i>px/sp*b*ca*h</i>	-0.064	0.059
<i>px/sp*b*e/sr*h</i>	0.148	< 0.0001
<i>b*ca*e/sr*h</i>	-0.134	< 0.0001
<i>px/sp*b*ca*e/sr*h</i>	0.042	0.22

cluding *h* in Table 2 would have the same magnitude and *P*-value, but with an opposite sign of effect. With this alternative interpretation of *h*, all significant interaction terms *not* involving *h* are negative, but five of the seven significant interactions including *h* are positive, implying diminishing returns epistasis. Given this difficulty of interpreting the effects of the regions marked by *h*, we may have more confidence in the results excluding *h*, in which case all significant interactions are negative (implying synergistic epistasis).

Although we cannot assign a *P*-value to the regression

TABLE 3. Logistic regression of male mating success on genotype.

Source	Likelihood-ratio χ^2	P	Estimate
<i>px/sp</i>	2.125	0.1449	0.139
<i>b</i>	28.742	0.0000	-0.496
<i>ca</i>	23.445	0.0000	-0.458
<i>e/sr</i>	50.339	0.0000	-0.665
<i>h</i>	3.213	0.0731	-0.169
<i>px/sp*b</i>	0.069	0.7927	-0.022
<i>px/sp*ca</i>	3.459	0.0629	-0.155
<i>px/sp*e/sr</i>	1.221	0.2691	0.102
<i>px/sp*h</i>	0.31	0.5776	0.045
<i>b*ca</i>	0.067	0.7961	0.024
<i>b*e/sr</i>	2.666	0.1025	-0.154
<i>b*h</i>	7.266	0.0070	-0.229
<i>ca*e/sr</i>	8.175	0.0042	-0.274
<i>ca*h</i>	5.153	0.0232	0.192
<i>e/sr*h</i>	2.889	0.0892	-0.162
<i>b*ca*e/sr</i>	10.065	0.0015	0.298

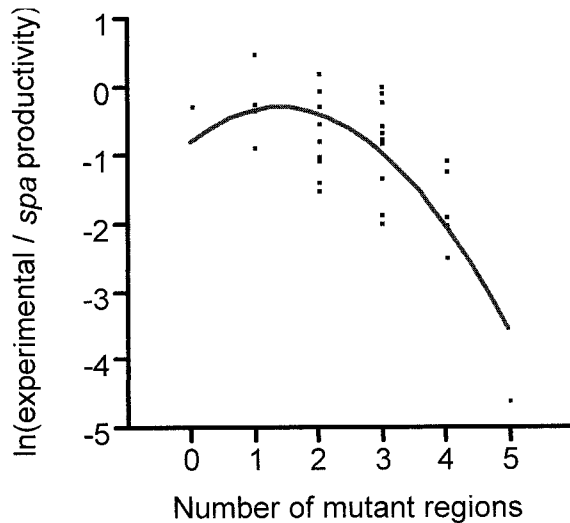


FIG. 1. The productivity of genotypes is a synergistic function of the number of mutations they carry. The best-fit prediction of the log-relative productivity of a line with x mutation regions is given by $\ln(\text{relative productivity}) = -0.80 + 0.72x - 0.25x^2$.

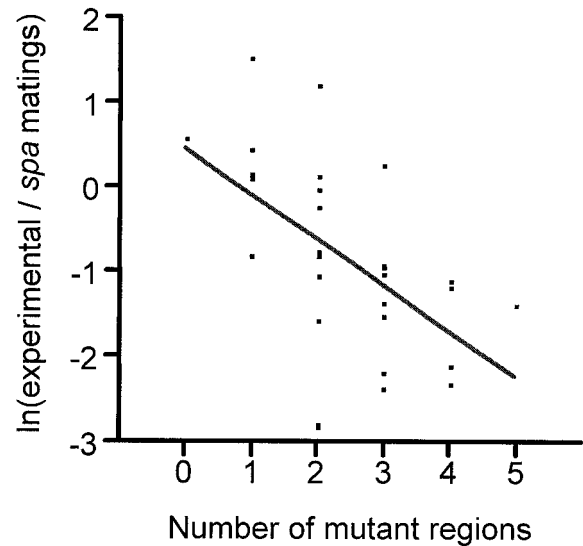


FIG. 2. The male mating success of a genotype is a nearly additive function of the number of mutations it carries. The best regression line is $\ln(\text{mating ratio}) = 0.46 - 0.54x$.

because of the nonindependence of the different genotypes, a quadratic regression of log-relative productivity against the number of mutation regions carried by a line shows a strong, negative quadratic term, as expected under the hypothesis of synergistic epistasis (Fig. 1). The best-fit prediction of the log-relative productivity of a line with x mutation regions is given by $\ln(\text{relative productivity}) = -0.80 + 0.72x - 0.25x^2$. The negative curvature of this relationship reappears in every one of 1000 resampled datasets created by sampling, with replacement, an equivalent number of measurements of productivity for each line, suggesting that the curvature is not due to measurement error in characterizing any particular line.

There is no evidence for directional epistasis in the interactions of loci determining relative male mating success (Fig. 2, Table 3). The logistic regression shows that only four of the 26 possible interaction terms are significantly different from zero, and these four are not consistent in direction (see Table 3). A linear regression of log-relative male mating success on number of mutations shows a strong linear decline with the number of mutations ($\ln[\text{relative mating success}] = 0.46 - 0.54x$; Fig. 2).

The Genetic Relationship between Mating Success and Productivity

Three of the four mutations with deleterious effects on productivity also have strong negative effects on male mating success. The fourth, *px/sp*, has no significant effect on mating success. The correlation between the estimates of the main effects of these mutations on the two traits is estimated to be high ($r = 0.6$), but is not significantly different from zero with this small sample size ($P = 0.29$; see Fig. 3).

DISCUSSION

These data show that recessive visible mutations in *D. melanogaster* are generally deleterious in a homozygous state,

that these deleterious effects can interact synergistically in determining productivity, and that alleles with effects on productivity are likely to also have deleterious effects on male mating success. These results are consistent with the notion that the genetic load in sexual populations should be less than that predicted by measuring the effects of single alleles on productivity alone.

Synergistic Epistasis

This study offers more data in the cumulative effort to assess the likelihood of synergistic epistasis. In the present case, a population that had all five of these mutation regions

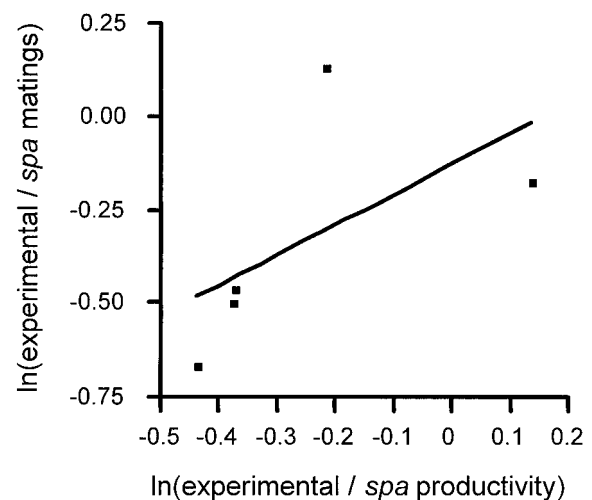


FIG. 3. Male mating success is low in most genotypes with low productivity. Each point represents the main effects of a mutation region on mating success and productivity. The line is the best-fit regression of mating success on productivity, but the slope is not significantly different from zero with this small sample size ($r = 0.6$, $P = 0.29$).

was much less productive relative to the fitness predicted by the effects of each of these loci alone. The magnitude of this epistasis seems to be relatively large. The productivity of the all-mutant combination is less than 4% of what would be expected under the multiplicative model based on the fitness of the other mutant lines.

The present data suffers from the fact that it measures the effects of only five mutation regions. Here these regions were viewed as fixed effects; as such, the analysis describes only the effects of these loci and does not strictly predict epistasis in general. A great deal of theoretical literature rests on the single datapoint of Mukai (1969), which gives some evidence for the presence of synergistic epistasis in novel mutations in *Drosophila*; this new experiment reinforces the possibility that this synergism is more general.

Further complicating the interpretation of these data is that one of the marked regions had a fitness larger than in the wild-type control (*h*). As such, it is difficult to assess whether it is more appropriate to use the mutant or wild-type region as the "mutant" strain. We are trying to assess the fitness interactions of deleterious mutations, thus, the simplest interpretation may be to focus more strongly on the other four mutation regions. For these four other mutations, there is a consistent pattern of synergistic epistasis for productivity in cases where there is evidence for interactions at all. This remains true when we look at the overall patterns of interactions and when the two subsets of the data that include *h*⁺ or *h*¹ strains alone are analyzed (results not shown).

One other caveat for a more general interpretation of these data is that these alleles are a potentially biased set relative to all possible mutations that affect fitness. The alleles cause visible effects on phenotypes; as such, they are likely to be stronger in effect than the average new mutation. It is unclear how this should affect our interpretations.

The present data, as well as previous experiments, are concerned with randomly chosen mutations that may not interact directly with one another and therefore may have lower probability of detecting strong epistasis (Rice 1998). A more powerful approach would be to look at closely interacting loci such as those involved in developmental or enzymatic pathways. Negative epistasis between such interacting loci may strongly reduce the mutation load and hence provide a large advantage to sexual recombination even in the absence of epistasis between mutations from different pathways (Rice 1998). The current data, however, give a better idea of the average level of epistasis expected among randomly chosen loci.

In some ways this present study has some desirable experimental design features. First, this is a direct measure of the fitness of specific genotypes; no indirect inference from other measures expected to be correlated with mutation number is required. Second, the experimental design prohibits selection from affecting the number of mutations displayed by these lines. All of the combinations of the various mutations are represented in the final dataset. Therefore, this improves on the designs that either do not capture all known combinations (and thereby risk losing the worst genotypes most likely to give evidence of synergism) as well as those designs that require differential numbers of generations to create the experimental material (such as the inbreeding stud-

ies, which also allow selection to purge the worst genotypes). As a result, we have an unbiased estimate of synergistic epistasis. The phenomenon may be more common than would be inferred from other recent studies. In the present case, for example, the worst genotype was difficult to maintain and could have been easily lost (and in fact has gone extinct since the experiments reported here). Without it, we would have seen much less evidence of synergistic epistasis. This sort of interline selection can be a very important problem for the study of epistasis for fitness.

Covariance of Fitness Components

These data show that alleles with deleterious effects on the productivity of a population can also cause a reduction in the mating success of males carrying the allele. This is significant, because in a population with the potential for polygyny, selection against males carrying an allele will be effective at reducing the frequency of that allele, without reducing the overall mean fitness of the population. As a result, deleterious alleles will be kept at lower frequency by stronger selection, but the overall effect on the population is less than it would be if selection acted via survivorship and female fecundity alone. Theoretical analyses of load assume that the strength of selection against an allele is the same as the effect of that allele on reducing the mean fitness of the population, and this is clearly not the case when part of the selection is on male mating success.

Given the nearly one-to-one correspondence between the strength of selection for productivity and the strength of selection on male mating success, it is possible that as much as half of the selection against these alleles is due to their effects on male mating success. If this were the case, then the mutational load, as measured on the effects on mean productivity, would be reduced by almost a factor of two. On average the drift load would be even more reduced, because the probability of fixation of deleterious alleles increases much faster than linearly with the strength of the selection coefficient (Crow and Kimura 1970).

This study has shown that there is at least the potential for strong synergistic epistasis among some alleles and that the deleterious effects of these alleles on the mean fitness of a population can be somewhat ameliorated by their correlated effects on male mating success. Both of these factors can affect the mutation load in a sexual population, but neither acts in an asexual population. The mutational deterministic hypothesis for the evolution of sex thus receives some support from these data.

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