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SHORT PAPER

The effect of synergistic epistasis on the inbreeding load

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Summary

The magnitude of inbreeding depression in *Drosophila melanogaster* appears too large to be accounted for by mutational load with multiplicative fitness interactions among loci, if current estimates of mutation and selection parameters are valid. One possible explanation for this discrepancy is synergistic epistasis among the fitness effects of deleterious mutations. A simple model of the effect of synergistic epistasis on the inbreeding load is developed. This model is used to show that deleterious mutations could account for the *Drosophila* data on the effects of inbreeding on components of fitness such as viability.

The purpose of this note is to examine the question of whether mutation–selection balance is sufficient to explain the effects of inbreeding on *Drosophila melanogaster*. Numerous experiments on the effects of inbreeding on components of fitness have been performed in this species, using balancers to extract chromosomes from random-bred populations (Simmons & Crow, 1977). The balancer procedure allows comparisons of the values of fitness components for chromosomal homozygotes and heterozygotes. The inbreeding load, B, is conveniently measured as the difference between the logarithms of the mean values of fitness components for chromosomal heterozygotes and homozygotes (Greenberg & Crow, 1960). A large number of measurements of B have been made for egg–adult viability, but more limited data are also available for traits such as female fecundity, male mating success, longevity and net fitness (Simmons & Crow, 1977; Crow & Simmons, 1983; Mukai, 1988; Charlesworth & Hughes, 1998). Using this technique, the component of the load due to genes with relatively small effects can be separated from that due to genes which confer complete inviability or sterility when homozygous (Greenberg & Crow, 1960). This 'detrimental load' is the subject of this note.

Under the hypothesis that inbreeding load is due to rare deleterious alleles maintained by a balance between mutation and selection, we can represent the fitness effects of variants at a given mutable site, i, in terms of the relative fitnesses of the three genotypes A_i A_i, $A_i\,a_i$ and $a_i\,a_i$, where A_i is the wild-type allele at the site, and a_i is the mutant allele. These fitnesses are conventionally designated as 1, $1-h_i s_i$ and $1-s_i$, respectively (Crow, 1970). A considerable body of evidence suggests that most detrimental mutations are not completely recessive (Simmons & Crow, 1977; Crow & Simmons, 1983; Charlesworth & Hughes, 1998).

The nature of the data can be illustrated by results for the third chromosome of *D. melanogaster*, for which the mean B estimate for detrimentals is 0·28 for viability (Simmons & Crow, 1977, table 2), and 0·19, 0·50, 0·09 for early and late male mating success, and male longevity, respectively (Hughes, 1995, table 1). For first male sperm precedence and second male sperm precedence, B values of 0·29 and 0·40 were obtained by Hughes (1997). The mean B over all these traits is 0·30, or 0·27 if sperm precedence is excluded, as may be warranted because of the unusual properties of its population statistics that suggest contributions from polymorphisms maintained by selection (Hughes, 1997). Two estimates of the inbreeding effects on net fitness for chromosome 3 gave a mean B value of 1·72 (Tracey & Ayala, 1974; Sved, 1975).

The observed B values for fitness components are generally much larger than is predicted by mutation–selection balance, if multiplicative fitness interactions and current estimates of mutation and selection parameters are assumed, and if the fact that the effect of a mutation on a given fitness component is likely to

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Table 1. *Log means of fitness components with epistasis*

Heterozygous for both chromosomes	$-\ln \bar{z} \approx 2\alpha h(\bar{n}_2+\bar{n}_3)+2\beta h^2(\bar{n}_2+\bar{n}_3)^2$
Homozygous for chromosome 2	$-\ln \bar{z} \approx \alpha(\bar{n}_2+2h\bar{n}_3)+\frac{1}{2}\beta(\bar{n}_2+2h\bar{n}_3)^2$
Homozygous for chromosome 3	$-\ln \bar{z} \approx \alpha(\bar{n}_3+2h\bar{n}_2)+\frac{1}{2}\beta(\bar{n}_3+2h\bar{n}_2)^2$
Homozygous for both chromosomes	$-\ln \bar{z} \approx \alpha(\bar{n}_2+\bar{n}_3)+\frac{1}{2}\beta(\bar{n}_2+\bar{n}_3)^2$

be only a fraction of its effect on fitness is taken into account (Charlesworth & Hughes, 1998). The discrepancy is particularly large for net fitness, for which Charlesworth and Hughes predicted a B of only 0·48 for chromosome 3. One possible explanation for the large B for net fitness is that some traits that contribute to net fitness (such as sperm precedence) may have alleles maintained by balancing selection. Alternatively, the mutation rate to deleterious alleles could be much larger than was assumed by Charlesworth and Hughes. This is, however, contrary to recent suggestions that this mutation rate has been overestimated (Keightley, 1996; Keightley & Caballero, 1997).

Another possibility is that the effects of mutations on net fitness may be more recessive than their effects on individual fitness components. Sved & Wilton (1989) showed that the data on net fitness can be explained by mutation–selection balance and multiplicative fitnesses if mean h values for fitness are less than 1/16, which is much smaller than the value suggested by the data on most fitness components (Crow & Simmons, 1983; Charlesworth & Hughes, 1998). Watanabe & Ohnishi (1975) reported a B value of 0·54 for a measure of the female fertility of non-sterile second chromosome homozygotes, which is much greater than is typical for fitness components. This suggests that female fertility may be an important contributor to the load for net fitness. It is interesting that their estimate of mean h_i for fertility was only 0·075, with an upper confidence limit of 0·17, which suggests a greater degree of recessivity for alleles affecting female fertility than for other life-history components (see below). This is consistent with the relatively high B of 0·48 for net fitness effects of the X chromosome (Wilton & Sved, 1979), which contrasts with the near-zero load for viability for this chromosome (Eanes *et al*., 1985). Such a difference would be expected if there is a substantial contribution to net fitness from fertility genes with female-limited effects.

Finally, synergistic epistasis between the effects of mutations could considerably increase the predicted inbreeding load (Sved & Wilton, 1989). This question can be examined using the model proposed by Sved & Wilton (1989) and Charlesworth *et al*. (1991). Consider first the simple case when all loci have the same effects. The value of a given fitness component of an individual is assumed to be an exponential quadratic function of the effective number, n_e, of mutations which it carries. In calculating n_e, the number of heterozygous mutations is weighted by the dominance coefficient h and added to the number of homozygous mutations (Sved & Wilton, 1989; Charlesworth *et al*., 1991). The fitness component value, z, is then given by

$$z = \exp -\{n_e(\alpha+\tfrac{1}{2}\beta n_e\}, \quad (1)$$

where α and β are positive constants.

Two types of experiments have been done to estimate B in *Drosophila* (reviewed by Simmons & Crow, 1977; Charlesworth & Hughes, 1998). Most commonly, a set of single major autosomes (chromosomes 2 or 3 in the case of *D. melanogaster*) are extracted from a population and made homozygous by the use of a balancer chromosome; the values of fitness components measured on the chromosomal homozygotes are compared either with the measurements for heterozygotes for the balancer and the extracted chromosomes, or with values for heterozygotes for pairs of randomly selected wild-type chromosomes. In this case, the chromosomal background for the unmanipulated chromosome is largely derived from the balancer stock, and approximates that of a random-bred population. In some cases, crosses using balancers for two chromosomes are used, and homozygotes for both autosomes as well as homozygotes for either autosome separately are characterized. This enables interaction effects between loci on the two autosomes to be estimated, as well as the B value for the chromosome in question (Spassky *et al*., 1965; Temin *et al*., 1969; Kosuda, 1971; Seager & Ayala, 1982).

In the majority of cases, the estimator of B for a single chromosome is the difference between the natural logarithms of the mean value of the traits for chromosomal heterozygotes and homozygotes, respectively (Greenberg & Crow, 1960; Simmons & Crow, 1977). The case of chromosomes 2 and 3 in *D. melanogaster* will be considered here (the contribution of the X chromosome will be ignored, as it seems to show little genetic load for viability: Eanes *et al*., 1985). Theoretical values for these can be derived as follows.

Let $\bar{n}_i$ and V_i be the population mean and variance, respectively, of the numbers of deleterious mutations carried on haploid chromosomes of type i ($i = 2$ or 3). In a random mating population, the mean and variance for diploid genotypes with respect to chromosome i are $2\bar{n}_i$ and $2V_i$. Using the approach of Charlesworth *et al*. (1991), expressions for the mean

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value of a given fitness component for populations of chromosomal heterozygotes, homozygotes for a single autosome, and homozygotes for both autosomes can be obtained by assuming a bivariate normal distribution of mutation numbers on chromosomes 2 and 3, with zero correlation coefficient. Unless synergistic selection is extremely strong, V_i is close to $\bar{n}_i$ (Charlesworth, 1990; Charlesworth & Barton, 1996). Provided that $\beta V_i \ll 1$, the contribution of variance terms to the expressions for the population means, can be neglected, yielding the approximations shown in Table 1. The inbred loads for chromosomes 2 and 3, respectively, are then

$$B_2 \approx \alpha\bar{n}_2(1-2h)+\tfrac{1}{2}\beta\{\bar{n}_2^2(1-4h^2)+4\bar{n}_2\bar{n}_3 h(1-2h)\}, \quad (2a)$$

$$B_3 \approx \alpha\bar{n}_3(1-2h)+\tfrac{1}{2}\beta\{\bar{n}_3^2(1-4h^2)+4\bar{n}_2\bar{n}_3 h(1-2h)\}. \quad (2b)$$

The interaction load, which measures the difference between the sum of the loads for each chromosome and the load for the double homozygotes (Spassky *et al*., 1965; Temin *et al*., 1969), is

$$i \approx \beta\bar{n}_2\bar{n}_3(1-2h[1-2h]). \quad (3)$$

Equations (2) enable us to examine the extent of synergetic epistasis needed to account for the observed values of B, by means of the following argument. Crow (1970) used the data of Mukai (1969) from a long-term mutation accumulation experiment to estimate the coefficients α and β for the effects of detrimental mutations on viability, and found that $\alpha \approx 0{\cdot}01$, $\beta \approx 0{\cdot}02$. Keightley (1996) has suggested that the curvilinearity of the regression of viability on time in Mukai's data may reflect the mobilization of transposable elements, and a resulting accelerated decline in viability due to insertional mutations, rather than synergistic epistasis. For this reason, it seems preferable to use the following method to estimate β, rather than employing Crow's estimate. If the $\bar{n}_i$ and h are known in addition to α, we can rearrange (2) and (4) to estimate the value of β for a given fitness component from empirical values of the B_i (note that (2) are valid for any fitness-related trait for which α is known, not just for net fitness). We have

$$\hat{\beta}_2 = \frac{2\{B_2-\alpha\bar{n}_2(1-2h)\}}{\bar{n}_2(1-2h)\{\bar{n}_2(1+2h)+4\bar{n}_3 h\}}, \quad (4a)$$

$$\hat{\beta}_3 = \frac{2\{B_3-\alpha\bar{n}_3(1-2h)\}}{\bar{n}_3(1-2h)\{\bar{n}_3(1+2h)+4\bar{n}_2 h\}}. \quad (4b)$$

The $\bar{n}_i$ can be estimated as follows. It is possible to define an effective mean selection coefficient, t, against a heterozygous mutant allele for an equilibrium random-mating population under synergistic selection, by the relation $\bar{n}_i = U_i/t$, where U_i is the haploid mutation rate for deleterious alleles for the chromosome in question (Charlesworth *et al*., 1991). t can be equated to the harmonic mean of hs derived from comparisons of mutational variances or declines in mean with corresponding population statistics (Crow & Simmons, 1983; Charlesworth & Hughes, 1998). The data suggest a t value of 0·01 to 0·02.

Assume that $U_2 = 0{\cdot}20$ and $U_3 = 0{\cdot}24$, as suggested by the second chromosome mutation accumulation experiments of Mukai and co-workers and the relative sizes of the second and third chromosomes (Crow & Simmons, 1983; Charlesworth & Hughes, 1998). Setting $t = 0{\cdot}02$, we have $\bar{n}_2 = 10$ and $\bar{n}_3 = 12$. The most extensive data on inbred loads are for egg-to-adult viability, summarized in table 2 of Simmons & Crow (1977). For detrimental mutations, the mean values of B_2 and B_3 over a large number of experiments are 0·236 and 0·284, respectively. Assuming that $h = 0{\cdot}25$, which is close to the mean value for all fitness components (Charlesworth & Hughes, 1998), we obtain $\beta_2 = 0{\cdot}00266$ and $\beta_3 = 0{\cdot}00267$, in remarkable agreement with each other. Setting $\beta = 0{\cdot}0027$, (4) yields a predicted value of the interaction load, i, of 0·24, i.e. i is of similar magnitude to the main effects.

This prediction can be compared with experimental measurements of i. Unfortunately, these have yielded rather disparate conclusions. In a series of experiments on chromosomes 2 and 3 of *D. pseudoobscura*, Spassky *et al*. (1965) found the mean of the interaction effects to be similar to the main effects, as did Kosuda (1971) in an experiment on *D. melanogaster*. Temin *et al*. (1969) found the interaction term to be much smaller than the main effects, and only marginally significant. In all these cases, the load estimates were based on mildly detrimental chromosomes (i.e. those for which viability was greater than 50% of normal), whereas the B estimates above were based on all categories of non-lethal and semi-lethal chromosomes. The mean estimates of B for mildly detrimental genes for 0·134 and 0·148 for chromosomes 2 and 3 respectively. Using the same parameters as above, the corresponding estimates of β are 0·00124 and 0·00105, giving a mean estimate of 0·0011. The predicted value of i is 0·099, again of a similar magnitude to the main effects. In contrast, Seager & Ayala (1982) obtained a marginally significant *negative* value of i from data on all classes of detrimental chromosomes. A possible source of bias in their experiment was pointed out by Charlesworth (1990).

Overall, the degree of epistasis required to explain inbred loads does not seem to be unreasonable in relation to the magnitude of interaction effects reported in the literature on the effects of making two autosomes homozygous simultaneously, but the uncertainty in the values of the parameters involved means that this conclusion must be regarded with some scepticism. It seems clear, however, that some degree of synergistic epistasis is required to fit the data if inbreeding effects are to be explained purely in terms of mutation and selection, unless deleterious muta-

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tions affecting viability are much more recessive than is indicated by the data. If β is set to zero in (2), the predicted values of B become 0·05 and 0·06 for chromosome 2 and 3 respectively. These are much smaller than even the mildly detrimental loads.

This raises the question of the robustness of the estimates of β to changes in the value of the parameters that appear in (3). The estimate of β is relatively insensitive to the value of α, since the estimate of β is much more heavily influenced by B_i than by α, unless $\bar{n}_i\alpha$ is comparable in magnitude to B_i. The estimates of β are, however, highly sensitive to $\bar{n}_i$; inspection of (2) shows that β is inversely proportional to $\bar{n}_i^2$ if $\bar{n}_i\alpha \ll B_i$. But i is proportional to $\beta\bar{n}_2\bar{n}_3$, and is thus not very sensitive to $\bar{n}_i$ when changes in β are accounted for. For example, if the values of $\bar{n}_i$ are doubled from those assumed above, the estimate of β from the total detrimental load for chromosome 2 data changes to $5{\cdot}0\times10^{-4}$ whereas the predicted value of i becomes 0·18 instead of 0·24. In addition, the estimated values of β and i are insensitive to a reduction in h. For example, if all the other parameters are held constant at their earlier values while h is changed to 0·15, we find that β and i become 0·0024 and 0·22 respectively.

This robustness suggests that the value of i that is required to epistasis to account for the inbreeding load for viability is fairly strongly constrained and should be measurable experimentally, if the basic model is valid. The model is, however, oversimplified in that it assumes equal effects of all loci with respect to their linear and quadratic effects on log fitness, as well as equal dominance coefficients. If there is variation among loci in α, β and h, (2) can be modified by treating the displayed parameters as means over loci, and adding appropriate covariance terms. This unfortunately yields a complicated sum, whose properties are difficult to evaluate.

It is possible, however, to ask whether the observed values of B can be explained by such variation in the absence of epistasis. In this case, the mean number of mutations per haploid genome for a set k of loci, all with selection parameters α_k and h_k, is proportional to the mutation rate for the set, divided by $\alpha_k h_k$, where the constant of proportionality is determined by the extent to which effects of the loci on net fitness are reflected in the fitness component in question (Charlesworth & Hughes, 1998). Assume that this constant is the same for all loci; furthermore, assume that the coefficient of dominance h_k for a given set of loci is related inversely to α_k, as indicated by several lines of evidence (Kacser & Burns, 1981; Crow & Simmons, 1983). A Taylor expansion of (2) then yields the following approximation:

$$B_i \approx \bar{n}_i\alpha\left\{(1-2h)+2h\alpha^2\left(\frac{\partial h}{\partial\alpha}\right)^2 C_\alpha^2\right\}, \quad (4)$$

where the partial derivative is evaluated at the mean of the α_k over all loci, and C_α is the coefficient of variation in α.

This shows that variation in the coefficient of dominance associated with variation in the magnitude of effects of loci on a fitness component can increase the value of B over the value predicted in the absence of such variation. The level of epistasis needed to account for observed values of B may be overestimated by the previous formulae. The effect is, however, likely to be small if $\alpha(\partial h/\partial\alpha)$ for detrimental mutations is $\ll 1$, as suggested by the fact that the mean dominance coefficient for new viability mutations ranges only from a value of approximately 0·02 for lethals to about 0·35 for detrimentals (Crow & Simmons, 1983), while α ranges from 1 to approximately 0·01. The conclusion that synergistic epistasis is needed to explain the observed loads is therefore likely to be robust to the existence of variation in the selection parameters.

This conclusion is reinforced by examining the effect of variation in the parameters on the estimated strength of epistasis in (3). Here, covariances between h_k, β_k and the mean numbers of mutations per haploid genome are all likely to be negative and generate additional terms of the form

$$2(4h-1)\{\mathrm{Cov}(\beta,h)+\mathrm{Cov}(\bar{n}_2\bar{n}_3,h)\}+\{1-2h(1-2h)\}\mathrm{Cov}(\bar{n}_2\bar{n}_3,\beta). \quad (6)$$

With an h of 0·25, as suggested above, the first two terms in (6) are zero, whereas the last term is negative. The observed value of i for a given mean level of epistasis is therefore reduced by variability in the selection parameters, so that (3) underestimates the mean value of β_k consistent with a given value of i. We can employ the formula for the equilibrium number of mutations under synergistic epistasis (Charlesworth, 1990) to obtain a first-order approximation for the effect of a change in β_k on the corresponding mean number of mutations per genome. The magnitude of the last term in (6) can then be shown to be substantially less than

$$\bar{n}_2\bar{n}_3\beta C_\beta\{1-2h(1-2h)\}.$$

This implies that the value of i is greatly reduced below that in (3) only if there is a very high level of variation in β_k.

These considerations suggest that it is at least possible that synergistic epistasis among the fitness effects of deleterious mutations contributes to the unexpectedly high level of inbreeding load observed in *Drosophila*, and that it may not be necessary to invoke contributions from loci maintained polymorphic by selection. As already discussed, other explanations cannot at present be ruled out. However, the difficulties for a mutational hypothesis in explaining observed levels of genetic variance, pointed out by

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Charlesworth & Hughes (1998), still apply. It is quite possible for additive genetic variance in a fitness component to have a substantial contribution from alleles maintained polymorphic by selection, whereas inbreeding depression is caused largely by rare deleterious alleles maintained by mutation. Inbreeding depression requires directional dominance, such that alleles reducing the trait value have a mean dominance coefficient of less than one-half, whereas there is no such requirement for additive genetic variance. Polymorphic loci with no dominance or with dominance in the wrong direction will therefore contribute to additive genetic variance but not to inbreeding depression.

A peculiarity of the results, however, is that the estimate of β for viability obtained here (0·0027) is much smaller than the value of 0·02 found by Crow (1970), when analysing Mukai's (1969) data. The discrepancy is probably too large to be accounted for purely by sampling error. In addition, the use of Crow's estimate in (3) produces a more than sevenfold increase in the predicted value of i, which is clearly inconsistent with the observed values. Keightley's (1996) suggest that the curvilinearity in Mukai's data may be at least partly artefactual should thus be considered seriously.

Given current uncertainties concerning the values of crucial parameters such as U (Keightley, 1996; Keightley & Caballero, 1997) and the extent of synergistic epistasis (Simmons & Crow, 1977; Charlesworth, 1990), the above conclusions are only tentative. Further data from mutation accumulation experiments are needed to resolve these questions.

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