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The genetic basis of inbreeding depression

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Summary

Data on the effects of inbreeding on fitness components are reviewed in the light of population genetic models of the possible genetic causes of inbreeding depression. Deleterious mutations probably play a major role in causing inbreeding depression. Putting together the different kinds of quantitative genetic data, it is difficult to account for the very large effects of inbreeding on fitness in *Drosophila* and outcrossing plants without a significant contribution from variability maintained by selection. Overdominant effects of alleles on fitness components seem not to be important in most cases. Recessive or partially recessive deleterious effects of alleles, some maintained by mutation pressure and some by balancing selection, thus seem to be the most important source of inbreeding depression. Possible experimental approaches to resolving outstanding questions are discussed.

1. Introduction

The decline in fitness components with inbreeding has attracted the attention of geneticists and evolutionary biologists since Darwin (Darwin, 1876; Wright, 1977, chap. 2; Thornhill, 1993; Falconer & Mackay, 1996, chap. 14). As was realized early after the rediscovery of Mendelian genetics, the deleterious effects of inbreeding reflect the consequences of increased homozygosity for alleles affecting fitness. Any genetic explanation for the phenomenon of inbreeding depression must be capable of accounting for the large magnitude of effects detected in outbreeding species of plants and animals (Charlesworth & Charlesworth, 1987; Husband & Schemske, 1995; Keller, 1998).

Two major hypotheses have long been discussed in the literature (reviewed by Wright, 1977). The first is the idea that inbreeding depression represents the effects of loci at which there is heterozygote advantage (overdominance), so that heterozygotes are superior to homozygotes. Since heterozygote advantage in fitness is a form of balancing selection, alleles will be maintained as polymorphisms, at least in randomly mating populations. The other classical hypothesis is that inbreeding depression is caused by recessive or partially recessive deleterious alleles; this is sometimes known as the dominance hypothesis (Wright, 1977). These alleles can be maintained either by balancing selection acting on their net fitness effects, or by recurrent mutation which balances the elimination of strictly deleterious alleles.

Despite almost a century of research on the genetic basis of inbreeding depression, the question of the relative importance of overdominance versus partial recessivity is still open to debate. Our main conclusion is that inbreeding depression in most fitness components probably reflects partial recessivity of the deleterious effects of alleles, rather than overdominance; mutation is probably a major cause of the maintenance of these alleles, but balancing selection may play a role as well. For certain traits, however, the *Drosophila* data are consistent with a major role of overdominance (Charlesworth & Hughes, 1999). Mutation to deleterious alleles is, therefore, probably not the sole source of the variation that underlies inbreeding depression. The mutational model is, however, a useful null hypothesis, whose rejection forces one to consider alternatives.

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2. Modelling inbreeding depression

We begin with a simple theoretical framework for modelling the effect of inbreeding on a fitness component, and then apply this to various types of empirical evidence. Our concern is primarily with interpreting the effects of inbreeding on the mean of a set of lines produced from a natural population by a defined set of matings, not with the genetic properties of individual inbred lines or crosses between them – the subject matter of biometrical genetics (Kearsey & Pooni, 1996).

It is important to distinguish between fitness components and fitness. Darwinian fitness can be measured only in certain laboratory populations of *Drosophila*, *Escherichia coli* or yeast, using genetic markers to monitor changes in marker genotype frequencies that indicate the effects of selection (Latter & Sved, 1994; Hartl & Clark, 1997). Most data on the effects of inbreeding therefore come from measurements of components of fitness, such as viability, female fecundity or male mating success. The models described below deal with fitness components; results for net fitness will be discussed where appropriate.

For a given fitness component, z, the mutation–selection balance model can be formulated as follows (Charlesworth & Hughes, 1999). Assume that genotypic differences in z are directly proportional to the corresponding differences in fitness, with a constant of proportionality α_i for the ith locus at which mutations affecting the trait can arise. Mutant alleles reduce trait values compared with wild-type, and are held at low frequencies, given by the standard formulae for mutation–selection equilibrium in a large randomly mating population (Haldane, 1927). Fitness may be affected by several traits controlled by the same locus, so that the net effect of a locus on fitness is the sum of its effects on each trait, weighted by their proportional contributions to fitness. There is empirical evidence for multiple effects of deleterious mutant alleles on fitness components in *D. melanogaster* (Crow, 1993; Houle *et al.*, 1994).

A similar model can be used when variation is maintained by balancing selection with either pure overdominance, such that genotypic differences in z are directly proportional to the corresponding differences in fitness, or directional selection on fitness components influenced by a given locus (Charlesworth & Hughes, 1999). Balancing selection maintaining intermediate allele frequencies can arise from directional selection if there is antagonistic pleiotropy, so that the two homozygotes at a locus differ in opposite directions either for different traits or for different environments (Rose, 1982; Charlesworth & Hughes, 1999).

Population genetic models of these three possibilities can readily be formulated when different loci affect fitness components independently, i.e. multiplicatively (Charlesworth & Hughes, 1999). The effect of inbreeding a population to an inbreeding coefficient of F can then be measured in terms of the difference between the natural logarithms of the mean trait values for an outbred population (O) and the inbred population (I):

$$\ln \bar{z}_{\mathrm{O}} - \ln \bar{z}_{\mathrm{I}} = BF. \quad (1)$$

The coefficient B is the regression of log mean trait value on inbreeding coefficient; equivalently, B can be regarded as the reduction in log fitness associated with complete inbreeding. It is often referred to as the inbreeding load, or number of lethal equivalents (Morton *et al.*, 1956; Charlesworth & Charlesworth, 1987). Table 1 gives the theoretical values of B for the models described above, assuming that changes in allele frequencies do not occur during the course of inbreeding.

Table 1. *Inbreeding load B under three models of variability*

Mutation selection balance

$h_i > 0$: $\quad B = \sum_i u_i \left(\frac{1}{h_i} - 2\right) \alpha_i$

$h_i = 0$: $\quad B = \sum_i (\sqrt{(u_i s_i)} \alpha_i$

Directional selection on the fitness component z, *with balancing selection on fitness*

$$B = \sum_i p_i q_i (1 - 2h_i)\, \delta z_i$$

Pure heterozygote advantage

$$B = \sum_i \frac{s_i t_i \alpha_i}{(s_i + t_i)}$$

The effect of homozygosity for allele a_i, relative to the trait value of homozygotes for A_i, is measured by δz_i.

The dominance coefficient, h_i, measures the extent to which the trait value is affected in heterozygotes (if $h_i <$ 0·5, a_i is partially recessive to A_i: recessivity is when $h_i =$ 0).

u_i is the mutation rate from A_i to a_i under the mutation–selection balance model.

p_i and q_i are the equilibrium frequencies of A_i and a_i under balancing selection.

3. Inferences from *Drosophila* experiments

(i) *Estimates of inbreeding load*

The largest single body of data concerning the effects of inbreeding on fitness components comes from experiments on *Drosophila*. Experiments in which genetically marked chromosomes with multiple inversions suppressing crossing over (balancers) are used to manipulate whole chromosomes are especially in-

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formative, since these can be used to produce flies which are homozygous for all the genes on a single chromosome extracted from a natural population, without any selection against deleterious alleles that could reduce the effects of inbreeding (Simmons & Crow, 1977). Results from other methods are reviewed by Latter & Sved (1994). Crosses between lines carrying independently isolated chromosomes can be performed to reconstruct an outbred population, and hence allow the determination of B for a single chromosome using (1). This technique also enables one to dissect the net inbreeding load into components due to homozygous lethal or sterile chromosomes, and that due to chromosomes with minor effects (detrimental chromosomes: see Simmons & Crow, 1977). Estimates of the net homozygous fitness effects of detrimental chromosomes can be obtained by competing extracted chromosomes against balancers in population cages (Sved, 1971; Latter & Sved, 1994).

The results of numerous experiments indicate that complete homozygosity for a single major autosome dramatically reduces the mean values of most fitness components. For the third chromosome of *D. melanogaster* (representing approximately 48% of the genome), the mean B estimate estimated from data on egg-to-adult viability is 0·28 for detrimental chromosomes (Simmons & Crow, 1977). Since approximately 40% of third chromosomes from nature carry lethals (Simmons & Crow, 1977), slightly more than 50% of the chromosome 3 inbreeding load for viability is due to lethals. Homozygosity for chromosome 2 reduces viability by a somewhat smaller amount, as expected from the smaller euchromatic DNA content of this chromosome (Heino *et al*., 1994). For early and late male mating success, and male longevity, respectively, B estimates of 0·19, 0·50 and 0·09 have been obtained for chromosome 3 (Hughes, 1995*a*), and first male and second male sperm precedence have B values of 0·29 and 0·40, respectively (Hughes, 1997). Two independent B estimates for net fitness for detrimental third chromosomes averaged 1·72 (Tracey & Ayala, 1974; Sved, 1975), much larger than for any single fitness component.

To determine whether these effects of inbreeding can be accounted for purely in terms of mutation–selection balance, or whether contributions from variation maintained by balancing selection must be invoked, we need estimates of mutation rates and dominance coefficients for substitution into the relevant equations in Table 1. It is clearly impractical to obtain information on the parameters of individual loci; instead, rather crude aggregate statistics must be employed.

(ii) *Estimates of deleterious mutation rates*

The spontaneous lethal mutation rate for chromosome 2 of *D. melanogaster* is approximately 0·005 per gamete per generation, approximately twice that for the X chromosome, as would be expected from their relative sizes (Crow & Simmons, 1983). The mutation rate for detrimental alleles is much more problematic. The classical results are due to Mukai and his co-workers (Mukai *et al*., 1972) and Ohnishi (1977), who accumulated spontaneous mutations on sets of second chromosomes sheltered from selection over many generations. Viability assays were periodically carried out on homozygous chromosomes extracted from these lines. A lower bound to the mean number of new detrimental mutations per second chromosome per generation was estimated from the ratio of the square of the per generation decline in mean due to mutation to the mutational increase in among-line variance (Bateman, 1959). The Mukai experiments suggested an average mutation rate of approximately 0·15 over several replicate experiments, whereas Ohnishi's estimate was approximately 0·06. If there is a wide distribution of effects of mutations, the Mukai results mean that the true detrimental mutation rate could be as high as 0·20 for chromosome 2 (Simmons & Crow, 1977).

Criticisms of these experiments have recently been made by Keightley (1996*b*, 1998); a recent replication of them (Fry *et al*., 1999) has yielded a much lower detrimental mutation rate for chromosome 2 (0·02), although a reanalysis of these data suggests that the mutation rate could be substantially higher (J. D. Fry, unpublished results). On the other hand, an independent measurement of the fitness decline of lines protected from selection was in good agreement with the Mukai results (Shabalina *et al*., 1997; but see Keightley, 1996*b*). This uncertainty makes the interpretation of data on inbreeding effects difficult. One possible resolution of these disagreements is that the detrimental mutation rate is indeed high, but that most mutations have such small effects on fitness components that they are not reliably detected in these experiments (Caballero & Keightley, 1998; Keightley, 1998).

(iii) *Estimates of mean dominance coefficients*

Under the mutational load model, the dominance coefficients, h_i, are critical for determining the expected level of inbreeding depression (Table 1). Data on the heterozygous effects of lethal second chromosomes isolated from natural populations indicate that lethals reduce viability only by 2–3% on average when heterozygous, with additional effects on other fitness components (Crow, 1993), so that a substantial inbreeding load can be caused by lethal mutations.

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Under the hypothesis of pure mutation–selection balance, mean dominance coefficients for detrimental mutations can be estimated in two ways. The first uses the square root of the ratio of the additive genetic variance to twice the variance among lines homozygous for haploid genomes sampled randomly from a population (Hughes 1995*a*; Deng & Lynch, 1996; Charlesworth & Hughes, 1999). The second uses the regression coefficient of the trait values for heterozygotes formed by crossing pairs of lines homozygous for independent single chromosomes on the sums of the parental lines' trait values (Mukai & Yamaguchi, 1974; Caballero *et al*., 1997; Charlesworth & Hughes, 1999). A similar approach can be used to estimate dominance coefficients for lines isolated from natural populations of highly inbreeding species (Johnston & Schoen, 1995). Both these methods provide estimates in which individual h_i values are weighted by $u_i \alpha_i^2 s_i$; the first estimate is equal to the geometric mean of the arithmetic and the harmonic weighted means of h_i; the second is simply the harmonic mean. Both estimates are expected to be zero under the pure overdominance model, and will not provide meaningful estimates of dominance coefficients if balancing selection due to antagonistic pleiotropy contributes substantially to variability (Charlesworth & Hughes, 1999).

In practice, the first estimate is about 0·12 for viability effects of mildly detrimental chromosomes (homozygous viabilities $> 50\%$ of normal), whereas estimates for most other fitness components are much higher ($\geqslant 0{\cdot}26$) (Charlesworth & Hughes, 1999). First and second male sperm precedence, however, give zero values (Hughes, 1997). The second estimate gives values of between 0·20 and 0·30 for viability effects of detrimental second chromosome effects, and 0·40 for detrimental third chromosomes (Charlesworth & Hughes, 1999). Hughes's (1995*a*) data for chromosome 3 gave a mean of 0·32 for early male mating success, late male mating success and male longevity when corrected for sampling bias (Caballero *et al*., 1997), and a measure of female fertility yielded a value of 0·075 (Watanabe & Ohnishi, 1975). These results seem to rule out pure overdominance as a major contributor to variation for traits other than sperm precedence, but not variation maintained by balancing selection due to antagonistic pleiotropy.

The arithmetic mean h_i (weighted by $u_i \alpha_i^2 s_i^2$) for newly arisen spontaneous mutations can be estimated by the regression method, using lines that have accumulated mutations. Mukai & Yamazaki (1968) obtained an estimate of about 0·40 for detrimental viability mutations on the second chromosome. This is much larger than the value for chromosomes from nature, as might be expected since it involves an arithmetic rather than a harmonic mean. Other data suggesting nearly intermediate dominance for detrimental viability mutations are reviewed by Caballero & Keightley (1994). As expected from metabolic control theory (Kacser & Burns, 1981; Keightley, 1996*a*), mutations of large effect (lethals) thus seem to be more nearly recessive than mutations with small effects on viability.

Houle *et al*. (1997) obtained a mean regression estimate of 0·12 for chromosome 2 detrimental mutations affecting early female fecundity, late female fecundity, male longevity, female longevity and early male mating success. This suggests that mutational effects on these traits differ from those for viability. Further investigation of this point is important.

(iv) *Comparisons of inbreeding loads with predicted values*

The estimated mutation and selection parameters can be related to the possible causes of inbreeding depression as follows, using the formulae in Table 1. For lethals, the estimated mutation rate of 0·005 per generation for the second chromosome (Section 3.ii) predicts a lethal inbreeding load of about 0·25, assuming a harmonic mean heterozygous effect of lethals on net fitness of 0·02. This is close to the observed value of the lethal inbreeding load (Simmons & Crow, 1977; Crow, 1993). Most of the lethal inbreeding load must be mutational in origin, given the low allelism rates among lethal chromosomes extracted from natural populations (Simmons & Crow, 1977). Since approximately half the inbreeding load is due to lethals, this implies a major contribution from mutational load to the total effect of inbreeding.

The picture is less clear for the detrimental component of the inbreeding load, especially given current uncertainty about detrimental mutation rates (see Section 3.ii). Relatively high values of the per chromosome mutation rate are assumed below, in order to be conservative. Assuming multiplicative fitness effects for a given fitness component, and independently distributed α_i, u_i and h_i, the following predictions for B can be derived from Table 1. With a mutation rate of 0·24 for chromosome 3, as suggested by the Mukai experiments (correcting for the relative sizes of the second and third chromosomes), and mean h_i of the order of 0·25 (Section 3.iii), the expected B for fitness is 0·48. With a mean $\alpha_i \approx 0{\cdot}37$ (as crudely estimated by Charlesworth & Hughes, 1999), the expected B for a fitness component is then 0·18. Comparisons of these predictions with observed values for several fitness components and for net fitness (Section 3.i) suggest that mutational variation explains only about 30% of the observed inbred load for net fitness, and about 60% of that for fitness components (Charlesworth & Hughes, 1999). This discrepancy cannot be resolved by invoking a high

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mutation rate to mutations with effects that are too small to be detected in mutation-accumulation experiments (Keightley, 1998; Caballero & Keightley, 1998); dominance coefficients for such mutations will probably be close to 0·5 (Kacser & Burns, 1981; Keightley, 1996*a*), so they would not contribute greatly to inbreeding load.

Alternatively, dominance coefficients for some traits may be much lower than for the examples discussed above (Sved & Wilton, 1989), so that equilibrium frequencies of mutant alleles would be higher than for the standard dominance coefficient estimates. For example, the *B* value of 0·54 for the female fertility of homozygous-fertile second chromosomes (Watanabe & Ohnishi, 1975) suggests that female fertility may be an important contributor to the detrimental load for net fitness. Alleles affecting female fertility appear to be more recessive than those for other fitness components (Section 3.iii). This is consistent with the relatively high inbred load of 0·48 for net fitness effects of the X chromosome (Wilton & Sved, 1979), in contrast to its near-zero load for viability (Eanes *et al*., 1985). Data on heterosis for fitness components in crosses between mutation-accumulation lines also suggest greater recessivity for female fertility and net fitness than for viability or male fertility (Fry *et al*., 1998).

Synergistic epistasis among the fitness effects of deleterious mutations (log fitness declining faster as the number of deleterious alleles carried by an individual increases: see Mukai, 1969; Crow, 1970; Kondrashov, 1988) can also greatly increase the inbreeding load over that predicated assuming multiplicativity, although it is still difficult to account for the very large net fitness load (Charlesworth, 1998). However, no consistent empirical support yet exists for strong enough synergism to account for inbreeding load under a purely mutational model (Charlesworth, 1998).

4. Results from quantitative genetic experiments on taxa other than *Drosophila*

(i) *Introduction*

No species other than *D. melanogaster* permits detailed quantitative genetic experiments of the kind just reviewed, but some simpler experimental designs are possible in other organisms. Data from other species, including plants, however, are of considerable importance because inbreeding depression is one of the major factors involved in the evolution of outcrossing rates (Jarne & Charlesworth, 1993; Uyenoyama *et al*., 1993).

(ii) *Classical quantitative genetic experiments*

Estimates of average dominance coefficients of genes affecting fitness components can be obtained from the components of genetic variance in the F2 and subsequent generations of crosses between pairs of inbred lines (Moll *et al*., 1964; Wright, 1977). Evidence for overdominance at individual loci has sometimes been found, but this cannot be taken as evidence for the presence of loci with true overdominance, as it is also explicable in terms of linkage to partially recessive alleles at other loci. When two strains of a plant or animal are crossed, any form of heterosis in the F1 generation will lead to apparent overdominance for any loci at which the strains have allelic differences, because of the linkage disequilibrium in the initial cross between loci affecting fitness and the loci whose genotypes are being scored. Allowing recombination over further generations should reduce or eliminate this effect, but true overdominance will persist. When tests have been done after several generations of recombination, rather than in the F2 generation, estimates of overdominance for new mutations show a consistent tendency to decrease (Schuler, 1954; Schuler & Sprague, 1955). The same is true in quantitative genetic experiments on maize based on back-crosses or later generations derived from line crosses, even when the F2 results suggest overdominance (reviewed by Moll *et al*., 1964; Wright, 1977). The results of such experiments on a number of plant species are unfavourable to the overdominance model (Kearsey & Pooni, 1996, p. 306; Dudash & Carr, 1998).

(iii) *Purging of deleterious alleles*

Experiments on very small or inbred populations can provide another source of information. Recessive or partially recessive deleterious mutations should be 'purged' from such populations, because mutant allele frequencies may be reduced more effectively by selection than in large randomly mating populations. This is because of the greater opportunity for selection against homozygotes for deleterious alleles, which are more frequent when progeny are generated by matings between individuals whose alleles have increased levels of identity by descent (Crow, 1970; Lande & Schemske, 1985; Charlesworth *et al*., 1990). Although the data are not yet conclusive, habitually inbreeding plant populations seem to have lower inbreeding loads than outbreeders (Husband & Schemske, 1995). This effect is not, however, decisive evidence for a predominance of mutational load in these populations, because it could be due either to purging of partially recessive deleterious mutations or to failure of highly inbreeding populations to maintain variability at loci

with overdominance for net fitness (Kimura & Ohta, 1971; Charlesworth & Charlesworth, 1995).

An experimental approach is therefore needed. Experiments involving replicate isolated, small populations or inbred lines should be capable of yielding evidence on whether purging of mutations can occur and to what extent fitness can be improved by this process (purging of deleterious mutations with major fitness effects can occur even by selection within lines, but its effects are most likely to be detectable when there is between-line selection as well: see Wang *et al*., 1999). The results of such experiments are, however, difficult to interpret, because fixation or loss of detrimental alleles will also occur due to genetic drift, so that the net effect may be little or no improvement in mean performance or reduction in inbreeding load (Hedrick, 1994; Wang *et al*., 1999). The larger the size of the inbred populations, the more effective is a given intensity of selection. In *D. melanogaster*, the fitness decline for a given inbreeding coefficient was less when lines were maintained with larger population sizes, compared with sib-mated lines (Ehiobu *et al*., 1989). This result cannot, however, easily be used to correct for effects of selection, because differences in population size also affect the number of generations needed to reach the same expected inbreeding coefficient, and hence the opportunity for selection. In addition, inbreeding coefficients may differ from their expected values under neutral transmission, because of selection at linked loci (Kimura & Ohta, 1971; Latter, 1998; Wang *et al*., 1999).

Various approaches allow purging of mutational load to be detected. Direct estimates of the mean values of fitness components in small or inbred populations are unlikely to detect fitness improvements in purging populations, because the higher homozygosity of purging populations leads to low fitness. Purging causes a reduction in the inbreeding load (Wang *et al*., 1999; Willis, 1999*b*), but loss and fixation of mutations also reduce inbreeding load without purging (Wang *et al*., 1999), since no reduction in fitness is caused by inbreeding unless there is allelic variation in the population. Purging of deleterious mutations is thus better detected by an increase in the mean performance of inbred genotypes isolated from the populations in question, when compared with genotypes with similar inbreeding coefficients isolated from outbred populations. Random allele frequency changes have no systematic effect on this statistic. In plants, such data can be obtained by self-fertilization; in *Drosophila*, they can be obtained using balancer chromosomes. A reduction in the inbreeding load for viability effects of chromosome 2 was observed in small populations of *D. melanogaster*, but was not, however, accompanied by consistent effects on the performance of inbred genotypes (Gilligan *et al*., 1997).

Another indication of purging of deleterious alleles is an increased mean performance of a set of lines produced by F1 crosses between replicate inbred populations. A mean net fitness greater than for the outbred population from which the inbred lines were started should not occur under balancing selection (Barrett & Charlesworth, 1991), but can be produced by purging of mutational load. Fitness components behave in a similar way to net fitness under the pure overdominance and mutational load models, but the expectation for a given fitness component under balancing selection with antagonistic pleiotropy is unclear. Consistent increases in the F1 means for a large set of fitness components would not, however, be expected under this model. Evidence for purging has been claimed for experiments on *D. subobscura* (Hollingsworth & Maynard Smith, 1955), the plant *Eichhornia paniculata* (Barrett & Charlesworth, 1991) and the butterfly *Bicyclus anynana* (Saccheri *et al*., 1996). Purging was also detected in a survey of data from zoo animals, in which fitness-related character values from animals having a documented history of inbreeding were higher than from sets of animals without such a history (Ballou, 1997).

In part, differences in results of different experiments may reflect the fact that deleterious alleles of major effect can readily be purged, while those with small selection coefficients may be almost impossible to purge in the absence of between-line selection (Charlesworth *et al*., 1990; Wang *et al*., 1999). Natural populations probably often contain mutant alleles with major fitness effects, so that data from the experiments just cited are likely to include a contribution from any major mutations that were present in the starting populations. Evidence is scanty on the distribution of fitness effects of mutant alleles in natural populations. The *Drosophila* data suggest a substantial contribution to inbreeding depression from large mutations (see Section 3.iv). Outcrossing populations of the plants *Mimulus guttatus* and buckwheat (*Fagopyrum esculentum*) have been shown to contain recessive alleles causing chlorophyll deficiency and male sterility, i.e. with major fitness effects. Allelism rates were low for these genes, consistent with this component of the genetic load being due to mutation at many loci, at rates similar to lethal mutation rates estimated from *D. melanogaster* (and providing no suggestion that these alleles are maintained in the populations by overdominance: Ohnishi, 1982; Willis, 1992). Although a substantial fraction of the inbreeding depression in male sterility in *M. guttatus* is attributable to deleterious alleles of major effect, most is not (Willis, 2000). Consistent with this, five generations of maintenance of lines by selfing single plants (during which a high proportion of lines failed), which should have purged most major mutations but not detrimentals, reduced inbreeding depression only

slightly, compared with the population before purging (Willis, 2000).

(iv) *Marker-based data*

A completely different source of information on the genetic basis of variation in fitness comes from datasets on marker loci, which allow inferences concerning the fitness effects of linked loci. Flowering plants offer one advantage over *D. melanogaster* for direct experimental investigations of the causes of inbreeding depression. Their generally larger number of chromosomes implies greater recombination rates, and this minimizes problems caused by linkage of markers to selected loci, which can result in apparent heterozygote advantage even when no overdominant loci are present.

(*a*) *Population level observations: heterozygosity–fitness component correlations*. Allozyme data from many different species show little evidence of overdominant selection. Strong overdominance would generate excess heterozygosity, detectable as significantly negative F_{IS} values, which are very rarely found in outcrossing populations (Brown, 1979; Mitton & Grant, 1984; Houle, 1989). In contrast, partially inbreeding populations often have higher heterozygote frequencies than expected, given the populations' breeding systems (Brown, 1979). This need not imply overdominance, however; in partially inbreeding populations, marker homozygotes are more homozygous than average at loci affecting fitness. The frequency of homozygotes should therefore be reduced by inbreeding depression for early mortality, leaving populations with excess heterozygotes at the adult stage, even for neutral markers (Brown, 1979). Older seeds are indeed significantly less homozygous than younger ones, in a variety of different species (e.g. Cheliak *et al*., 1985; Alvarez-Buylla & Garay, 1994; Cabin *et al*., 1998). These data are consistent with the hypothesis that alleles other than the markers are the cause of variability in fitness components. Latter (1998) concluded that the observed retardation of the approach to homozygosity at marker loci in small populations of *D. melanogaster* was due to the effects of many linked loci with small effects of fitness, rather than to selection at the marker genes themselves.

An hypothesis of such associative overdominance can account for the many published observations of correlations between heterozygosity at marker loci and fitness-related characters in populations of self-compatible organisms (reviewed by David, 1998). Most recent evidence tends to support an important role for recessive or partially recessive alleles in causing such associations, and to weaken the evidence for overdominance at the marker loci or even at closely linked loci (David, 1998). Data from populations whose levels of inbreeding were once assumed to be very low, such as marine bivalves (e.g. Zouros *et al*., 1980; Koehn & Gaffney, 1984), are now revealing higher than expected homozygote frequencies in these populations. In particular, bimodal distributions of heterozygosity in these hermaphrodite animal populations (e.g. Zouros & Foltz, 1984; Gaffney *et al*., 1990) suggest the possibility of some inbreeding. A very telling kind of evidence is that, when individuals having the same inbreeding coefficient have been compared, heterozygosity–fitness component relationships are weakened or abolished (Beaumont *et al*., 1983; Strauss, 1986; Leberg *et al*., 1990). These findings support the view that overdominance is often apparent, rather than really present at the single-locus level. More such data are needed, particularly from populations in which heterozygosity–fitness component correlations have been detected.

Statistical tests for distinguishing between true and pseudo-overdominance have not yet been widely used. Although the test originally proposed (Smouse, 1986) yields the same expectations for both models (Houle, 1994), new models and tests, employing data from multiple loci, may have the potential to resolve this question (David, 1998). If overdominance is frequently involved, different marker loci may be associated with effects on different fitness components, whereas this is less likely if the markers reflect differences in genome-wide homozygosity due to partial inbreeding. This can provide the basis for testing the different hypotheses, if large sample sizes are obtained.

A different test is based on the fact that, because extremely heterozygous individuals are rare, their trait values depend on chance aspects of their actual genotypes at neutral loci, and so it is possible for their values for a fitness component to be slightly lower than for less heterozygous genotypes. This was found in the only study that has monitored fitness for a large number of heterozygous loci (Strauss & Libby, 1987), consistent with the other tests that fail to detect true overdominance. Now that molecular markers can readily be developed for natural populations, it would be worthwhile to obtain further data of this kind.

(*b*) *Family data*. Data on individual families may provide better information than population-level correlations. They can give evidence on the fitness effects associated with individual marker loci, and thus potentially reflect the effects of individual loci subject to selection. If deleterious mutations are widespread in natural populations, segregation ratios at closely linked marker loci will be affected. In a set of 451 plants from all crosses between three different inbred *Arabidopsis thaliana* strains (ecotypes), eight of 190 molecular markers scored showed evidence of

directional selection. These are not, however, natural population data, and some strains had been subjected to mutagens, so that this may be an overestimate (Mitchell-Olds, 1995). The frequency of such effects (estimated to be 13%, based on within-species data on randomly chosen markers in three angiosperms: Zamir & Tadmor, 1986) suggests that many loci affecting fitness are segregating in natural populations of plants. This kind of situation can be used in a manner analogous to QTL studies of non-fitness characters to infer the presence of such loci (Hedrick & Muona, 1990), to estimate their dominance (Stuber *et al*., 1992; Fu & Ritland, 1993; Fu & Ritland, 1994*b*; Mitchell-Olds, 1995) and to detect epistasis between them. In the few available studies, using maize and *M. guttatus*, epistasis was estimated to be slight (Stuber *et al*., 1992; Fu & Ritland, 1996).

The models used to analyse these data are oversimplified, and assume only one locus affecting fitness linked to each marker locus, but models including two selected loci generally do not greatly affect the inference of dominance unless these are very closely linked to the marker, so that flowering plants, which often have chromosome numbers of 10 or more, may provide useful information (Fu *et al*., 1997). The conclusion thus seems robust that most of the loci that affect flowering time in *M. guttatus* have dominance coefficients below 0·5 (Fu & Ritland, 1994*a*). However, data for viability suggested dominance coefficients greater than 0·5 for the same species, implying that selection in the haploid stage may affect the transmission of markers and leading to overestimates of dominance (Fu & Ritland, 1993). This is known to happen in some cases (Fulton *et al*., 1997) and complicates marker-based inferences about dominance. Such selection could substantially reduce overall levels of inbreeding depression (Charlesworth & Charlesworth, 1992), exacerbating the problem of explaining the high inbreeding depression of outcrossing plants (Husband & Schemske, 1995). More data would be valuable, including direct tests for selection in the haploid stage. For any overdominant region found it should also be possible to test whether data from multiple independent families consistently show overdominance, as must occur with true overdominance. If no overdominant locus is actually present, only certain families will appear to show overdominance because of repulsion phase linkage disequilibrium between different detrimental mutations, since such linkage disequilibrium will not occur generally.

5. Discussion

The data and models that we have reviewed suggest the following set of conclusions. First, several lines of evidence from *Drosophila* and plants concur in suggesting that pure overdominance of allelic effects on individual fitness components is not commonly the basis for inbreeding depression, although it may be important for certain traits, and might therefore contribute to the very high inbreeding depression for net fitness observed in *Drosophila* and outcrossing plants. This conclusion is reinforced by the general predominance of additive genetic variance as opposed to dominance variance for most fitness components in *Drosophila*, again with certain exceptions such as sperm precedence (Charlesworth, 1987; Mukai, 1988; Houle, 1992, 1998; Charlesworth & Hughes, 1999). A predominance of additive genetic variance is not expected with pure overdominance (Haldane, 1949).

Secondly, the data on detrimental inbreeding loads are compatible with a significant contribution from alleles maintained polymorphic by balancing selection, provided that there are antagonistic pleiotropic deleterious effects of partially recessive alleles on different traits, or on the same trait in different environments. It is hard to account for the magnitude of inbreeding load for fitness components such as viability in *Drosophila* on the basis of the Mukai estimates of mutation and selection parameters for detrimental alleles (Mukai *et al*., 1972; Crow, 1993), unless there is also a good deal of synergistic epistasis, for which there is limited empirical support (Charlesworth, 1998). On a purely mutational model, the large inbreeding load for net fitness requires either a much higher detrimental mutation rate than is indicated even by the Mukai experiments, or much greater recessivity of mutational effects on net fitness than is typically observed for individual fitness components (Sved & Wilton, 1989). The lack of evidence for selective elimination of detrimental alleles in a *Drosophila* cage experiment (Wilton *et al*., 1989) also suggests a role for balancing selection. Further work on detrimental mutation rates and the level of dominance of detrimental mutations is necessary before firm conclusions can be reached.

In contrast, it seems clear that the inbreeding load due to lethals in *Drosophila*, which is roughly 50% of the total, is mutational in origin (Simmons & Crow, 1977; Crow & Simmons, 1983; Crow, 1993). Not enough is yet known about lethal mutation rates and allelism rates in other species to determine whether this conclusion can be generalized, although there is evidence for a significant contribution from alleles with major effects to the total inbreeding load in outcrossing species of flowering plants (Husband & Schemske, 1995; Willis, 1993, 1999*a*, *b*). Mutations with major effects are rapidly purged from small or inbred populations (Lande & Schemske, 1985; Hedrick, 1994; Wang *et al*., 1999), which fits the comparative evidence suggesting that there is much less inbreeding load associated with early-acting genes in highly selfing plant populations compared with out-

crossers, assuming that such genes predominantly have major effects (Husband & Schemske, 1995).

Thirdly, a possible role for balancing selection and antagonistic pleiotropy is supported by data on the amount of additive genetic variance for fitness components in *D. melanogaster* populations. The models presented above suggest that it is impossible to account for the overall level of additive genetic variance in traits such as viability purely on a mutational model, if the Mukai estimates of mutational parameters are used (Charlesworth & Hughes, 1999). Use of smaller mutation rates and/or selection coefficients, as suggested by Keightley (1996*b*, 1998), would exacerbate this discrepancy (Charlesworth & Hughes, 1999). Appeals to effects of epistasis or linkaged disequilibrium seem incapable of resolving it (Charlesworth & Hughes, 1999), strongly suggesting a role for balancing selection as well as mutation in the maintenance of variability in fitness components. This contradicts the conclusion of Houle *et al*., (1996) that deleterious mutations contribute the bulk of the additive genetic variance in fitness components, for reasons discussed by Charlesworth & Hughes (1999). If the likely pleiotropic effects of mutations on sets of fitness traits are taken into account (Crow, 1993; Houle *et al*., 1994), it is thus very difficult to account for observed levels of genetic variation and inbreeding depression purely in terms of mutation–selection balance. This casts doubt on methods for estimating mutation and selection parameters from simultaneous measurements of additive genetic variance, homozygous genetic variance and inbreeding depression in outbreeding species (Deng & Lynch, 1996; Deng, 1998; Deng & Fu, 1998; Li *et al*., 1999).

Finally, we note that other models of inbreeding depression have been proposed, in addition to those discussed above. The most popular is the idea of resource partitioning by different genotypes, such that a set of genetically variable progeny produced by an outcross lead to greater mean performance than a set of more uniform progeny produced by inbreeding (Schmitt & Ehrhardt, 1987). While this could be a contributory factor in experiments where, say, selfed and outcrossed progeny are compared, the *Drosophila* experiments using balancers ensure that cultures of both the outbred controls and the inbred chromosomal homozygotes are genetically uniform with respect to the chromosome under investigation, so that only the level of heterozygosity differs. The large effects of inbreeding in *Drosophila* cannot, therefore, be explained by this model. There is also little direct support for it from plant experiments (Schmitt & Ehrhardt, 1987; Argyres & Schmitt, 1992), although more data are needed.

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References

Alvarez-Buylla, E. R. & Garay, A. A. (1994). Population genetic structure of *Cecropia obtusifolia*, a tropical pioneer tree species. *Evolution* **48**, 437–453.

Argyres, A. Z. & Schmitt, J. (1992). Neighbor relatedness and competitive performance in *Impatiens capensis* (Balsaminaceae): a test of the resource partitioning hypothesis. *American Journal of Botany* **79**, 181–185.

Ballou, J. D. (1997). Ancestral inbreeding only minimally affects inbreeding depression in mammalian populations. *Journal of Heredity* **88**, 169–178.

Barret, S. C. H. & Charlesworth, D. (1991). Effects of a change in the level of inbreeding on the genetic load. *Nature* **352**, 522–524.

Bateman, A. J. (1959). The viability of near-normal irradiated chromosomes. *International Journal of Radiation Biology* **1**, 170–180.

Beaumont, A. R., Beveridge, C. M. & Budd, M. D. (1983). Selection and heterozygosity within single families of the mussel *Mytilus edulis* (L.). *Marine Biology Letters* **4**, 151–161.

Brown, A. H. D. (1979). Enzyme polymorphism in plant populations. *Theoretical Population Biology* **15**, 1–42.

Bush, R. M., Smouse, P. E. & Ledig, F. T. (1987). The fitness consequences of multiple-locus heterozygosity: the relationship between heterozygosity and growth rate in pitch pine (*Pinus rigida* Mill.). *Evolution* **41**, 787–798.

Caballero, A. & Keightley, P. D. (1994). A pleiotropic nonadditive model of variation in quantitative traits. *Genetics* **138**, 883–900.

Caballero, A. & Keightley, P. D. (1998). Inferences on genome-wide deleterious mutation rates in inbred populations of *Drosophila* and mice. *Genetica* **102/103**, 229–239.

Caballero, A., Keightley, P. D. & Turelli, M. (1997). Average dominance for polygenes: drawback of regression estimates. *Genetics* **147**, 1487–1490.

Cabin, R. J., Mitchell, R. J. & Marshall, D. L. (1998). Do surface plant and soil seed bank populations differ genetically? A multipopulation study of the desert mustard *Lesquerella fendleri* (Brassicaceae). *American Journal of Botany* **85**, 1098–1109.

Charlesworth, B. (1987). The heritability of fitness. In *Sexual Selection: Testing the Alternatives* (ed. J. W. Bradbury & M. B. Andersson), pp. 21–40. Chichester: Wiley.

Charlesworth, B. (1998). The effect of synergistic epistasis on the inbreeding load. *Genetical Research* **71**, 85–89.

Charlesworth, B. & Hughes, K. A. (1999). The maintenance of genetic variation in life-history traits. In *Evolutionary Genetics from Molecules to Morphology* (ed. R. S. Singh & C. B. Krimbas). Cambridge: Cambridge University Press (in the press).

Charlesworth, D. & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* **18**, 237–268.

Charlesworth, D. & Charlesworth, B. (1992). The effects of selection in the gametophyte stage on genetic load. *Evolution* **46**, 703–720.

Charlesworth, D. & Charlesworth, B. (1995). Quantitative genetics in plants: the effect of breeding system on genetic variability. *Evolution* **49**, 911–920.

Charlesworth, D., Morgan, M. T. & Charlesworth, B. (1990). Inbreeding depression, genetic load and the evolution of outcrossing rates in a multi-locus system with no linkage. *Evolution* **44**, 1469–1489.

Cheliak, W. M., Dancik, B. P., Morgan, K., Yeh, F. C. H.

& Strobeck, C. (1985). Temporal variation of the mating system in a natural population of jack pine. *Genetics* **109**, 569–584.

Crow, J. F. (1970). Genetic loads and the cost of natural selection. In *Mathematical Topics in Population Genetics* (ed. K.-I. Kojima), pp. 128–177. Berlin: Springer.

Crow, J. F. (1993). Mutation, mean fitness, and genetic load. *Oxford Surveys in Evolutionary Biology* **9**, 3–42.

Crow, J. F. & Simmons, M. J. (1983). The mutation load in *Drosophila*. In *The Genetics and Biology of Drosophila*, vol. 3c (ed. M. Ashburner, H. L. Carson & J. N. Thompson), pp. 1–35. London: Academic Press.

Darwin, C. R. (1876). *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom*. London: John Murray.

David, P. (1998). Heterozygosity–fitness correlations: new perspectives on old problems. *Heredity* **80**, 531–537.

Deng, H.-W. (1998). Characterizing deleterious genomic mutations in outcrossing populations. *Genetics* **151**, 945–956.

Deng, H.-W. & Fu, Y.-X. (1998). On the three methods for estimating deleterious genomic mutation parameters. *Genetical Research* **71**, 223–236.

Deng, H.-W. & Lynch, M. (1996). Estimation of deleterious-mutation parameters in natural populations. *Genetics* **144**, 349–360.

Dudash, M. & Carr, D. E. (1998). Genetics underlying inbreeding depression in *Mimulus* with contrasting mating systems. *Nature* **393**, 682–684.

Eanes, W. F., Hey, J. & Houle, D. (1985). Homozygous and hemizygous viability variation on the X chromosome of *Drosophila melanogaster*. *Genetics* **111**, 831–844.

Ehiobu, N. G., Goddard, M. E. & Taylor, J. F. (1989). Effect of rate of inbreeding on inbreeding depression in *Drosophila melanogaster*. *Theoretical and Applied Genetics* **77**, 123–127.

Falconer, D. S. & Mackay, T. F. C. (1996). *An Introduction to Quantitative Genetics*, 4th edn. Harlow, Essex: Longman.

Fry, J. D., Heinsohn, S. L. & Mackay, T. F. C. (1998). Heterosis for viability, fecundity and male fertility in *Drosophila melanogaster*: comparison of mutational and standing variation. *Genetics* **148**, 1171–1188.

Fry, J. D., Keightley, P. D., Heinsohn, S. L. & Nuzhdin, S. V. (1999). New estimates of the rates and effects of mildly deleterious mutation in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the USA* **96**, 574–579.

Fu, Y.-B. & Ritland, K. (1993). Evidence for the partial dominance of viability genes in *Mimulus guttatus*. *Genetics* **136**, 323–331.

Fu, Y.-B. & Ritland, K. (1994*a*). Marker-based inferences about the genetic-basis of flowering time in *Mimulus guttatus*. *Hereditas* **121**, 267–272.

Fu, Y.-B. & Ritland, K. (1994*b*). On estimating the linkage of marker genes to viability genes controlling inbreeding depression. *Theoretical and Applied Genetics* **88**, 925–932.

Fu, Y.-B. & Ritland, K. (1996). Marker-based inferences about epistasis for genes influencing inbreeding depression. *Genetics* **144**, 339–348.

Fu, Y.-B., Charlesworth, D. & Namkoong, G. (1997). Point estimation and graphical inference of marginal dominance for two viability loci controlling inbreeding depression. *Genetical Research* **70**, 143–153.

Fulton, T. M., Nelson, J. C. & Tanksley, S. D. (1997). Introgression and DNA marker analysis of *Lycopersicon peruvianum*, a wild relative of the cultivated tomato, into *Lycopersicon esculentum*, followed through three successive backcross generations. *Theoretical and Applied Genetics* **95**, 895–902.

Gaffney, P. M., Scott, T. M., Koehn, R. K. & Diehl, W. J. (1990). Interrelationships of heterozygosity, growth rate and heterozygote deficiencies in the coot clam. *Mulinia lateralis*. *Genetics* **124**, 687–699.

Gilligan, D. M., Woodworth, L. M., Montgomery, M. E., Briscoe, D. A. & Frankham, R. (1997). Is mutational meltdown a threat to the survival of endangered populations? *Conservation Biology* **11**, 1235–1241.

Haldane, J. B. S. (1927). A mathematical theory of natural and artificial selection. V. Selection and mutation. *Proceedings of the Cambridge Philosophical Society* **23**, 838–844.

Haldane, J. B. S. (1949). Parental and fraternal correlations in fitness. *Annals of Eugenics* **14**, 288–292.

Hartl, D. L. & Clark, A. G. (1997). *Principles of Population Genetics* 3rd end. Sunderland, MA: Sinauer.

Hedrick, P. W. (1994). Purging inbreeding depression and the probability of extinction: full-sib mating. *Heredity* **73**, 363–372.

Hedrick, P. W. & Muona, O. (1990). Linkage of viability genes to marker loci in selfing organisms. *Heredity* **64**, 67–72.

Heino, T. I., Saura, A. O. & Sorsa, V. (1994). Maps of the salivary gland chromosomes of *Drosophila melanogaster*. *Drosophila Information Service* **73**, 621–738.

Hollingsworth, M. J. & Maynard Smith, J. (1955). The effects of inbreeding on rate of development and on fertility in *Drosophila subobscura*. *Journal of Genetics* **53**, 295–314.

Houle, D. (1989). Allozyme-associated heterosis in *Drosophila melanogaster*. *Genetics* **123**. 789–801.

Houle, D. (1992). Comparing evolvability and variability of quantitative traits. *Genetics* **130**, 195–204.

Houle, D. (1994). Adaptive distance and the genetic basis of heterosis. *Evolution* **48**, 1410–1417.

Houle, D. (1998). How should we explain variation in the genetic variance of traits? *Genetica* **102/103**, 242–253.

Houle, D., Hughes, K. A., Hoffmaster, D. K., Ihara, J. T., Assimacopoulos, S. & Charlesworth, B. (1994). The effect of spontaneous mutation on quantitative traits. I. Variances and covariances of life history traits. *Genetics* **138**, 773–785.

Houle, D., Morikawa, B. & Lynch, M. (1996). Comparing mutational variabilities. *Genetics* **143**, 1467–1483.

Houle, D., Hughes, K. A., Assimacopoulos, S. & Charlesworth, B. (1997). The effects of spontaneous mutation on quantitative traits. II. Dominance of mutations with effects on life-history traits. *Genetical Research* **70**, 27–34.

Hughes, K. A. (1995*a*). The inbreeding decline and average dominance of genes affecting male life-history characters in *Drosophila melanogaster*. *Genetical Research* **65**, 41–52.

Hughes, K. A. (1995*b*). Evolutionary genetics of male life history characters in *Drosophila melanogaster*. *Evolution* **49**, 521–537.

Hughes, K. A. (1997). The quantitative genetics of sperm precedence in *Drosohila melanogaster*. *Genetics* **145**, 139–151.

Husband, B. C. & Schemske, D. W. (1995). Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**, 54–70.

Jarne, P. & Charlesworth, D. (1993). The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annual Review of Ecology and Systematics* **23**, 441–466.

Johnston, M. O. & Schoen, D. J. (1995). Mutation rates and

dominance levels of genes affecting total fitness in two angiosperm species. *Science* **267**, 226–229.
Kacser, H. & Burns, J. A. (1981). The molecular basis of dominance. *Genetics* **97**, 639–666.
Kearsey, M. J. & Pooni, H. S. (1996). *The Genetical Analysis of Quantitative Traits*. London: Chapman and Hall.
Keightley, P. D. (1996*a*). A metabolic basis for dominance and recessivity. *Genetics* **143**, 621–625.
Keightley, P. D. (1996*b*). The nature of deleterious mutation load in *Drosophila*. *Genetics* **144**, 1993–1999.
Keightley, P. D. (1998). Inference of genome-wide mutation rates and distributions of mutation effects for fitness traits: a simulation study. *Genetics* **150**, 1283–1293.
Keller, L. F. (1998). Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). *Evolution* **52**, 240–250.
Kimura, M. & Ohta, T. (1971). *Theoretical Topics in Population Genetics*. Princeton, NJ: Princeton University Press.
Koehn, R. K. & Gaffney, P. M. (1984). Genetic heterozygosity and growth rate in *Mytilus edulis*. *Marine Biology* **82**, 1–7.
Kondrashov, A. S. (1988). Deleterious mutations and the evolution of sexual reproduction. *Nature* **336**, 435–440.
Lande, R. & Schemske, D. W. (1985). The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**, 24–40.
Latter, B. D. H. (1998). Mutant alleles of small effect are primarily responsible for the loss of fitness with slow inbreeding in *Drosophila melanogaster*. *Genetics* **148**, 1143–1158.
Latter, B. D. H. & Sved, J. A. (1994). A reevaluation of data from competitive tests shows high levels of heterosis in *Drosophila melanogaster*. *Genetics* **137**, 509–511.
Leberg, P. L., Smith, M. H. & Rhodes, O. E. (1990). The association between heterozygosity and growth of deer fetuses is not explained by the effects of the loci examined. *Evolution* **44**, 454–458.
Li, J.-L., Li, J. & Deng, H.-W. (1999). The effects of overdominance on characterizing deleterious mutations in large natural populations. *Genetics* **151**, 895–913.
Mitchell-Olds, T. (1995). Interval mapping of viability loci causing heterosis in *Arabidopsis*. *Genetics* **140**, 1105–1109.
Mitton, J. B. & Grant, M. C. (1984). Associations among protein heterozygosity, growth rate, and developmental homeostasis. *Annual Review of Ecology and Systematics* **15**, 479–499.
Moll, R. H., Lindsley, M. F. & Robinson, H. F. (1964). Estimates of genetic variances and level of dominance in maize. *Genetics* **49**, 411–423.
Morton, N. E., Crow, J. F. & Muller, H. J. (1956). An estimate of the mutational damage in man from data on consanguineous marriages. *Proceedings of the National Academy of Sciences of the USA* **42**, 855–863.
Mukai, T. (1969). The genetic structure of natural populations of *Drosophila melanogaster*. VII. Synergistic interactions of spontaneous mutant polygenes affecting viability. *Genetics* **61**, 749–761.
Mukai, T. (1988). Genotype–environment interaction in relation to the maintenance of genetic variability in populations of *Drosophila melanogaster*. In *Proceedings of the 2nd International Conference on Quantitative Genetics* (ed. B. S. Weir, E. J. Eisen, M. M. Goodman & G. Namkoong), pp. 21–31. Sunderland, MA: Sinauer.
Mukai, T. & Yamaguchi, O. (1974). The genetic structure of natural populations of *Drosophila melanogaster*. XI. Genetic variability in a local population. *Genetics* **76**, 339–366.
Mukai, T. & Yamazaki, T. (1968). The genetic structure of natural populations of *Drosophila melanogaster*. V. Coupling-repulsion effect of spontaneous mutant polygenes controlling viability. *Genetics* **59**, 513–535.
Mukai, T., Chigusa, S. I., Mettler, L. E. & Crow, J. F. (1972). Mutation rate and dominance of genes affecting viability in *Drosophila melanogaster*. *Genetics* **72**, 335–355.
Mukai, T., Cardellino, R. A., Watanabe, T. K. & Crow, J. F. (1974). The genetic variance for viability and its components in a local population of *Drosophila melanogaster*. *Genetics* **78**, 1195–1208.
Ohnishi, O. (1977). Spontaneous and ethyl methanesulfonate-induced mutations controlling viability in *Drosophila melanogaster*. II. Homozygous effects of polygenic mutations. *Genetics* **87**, 529–545.
Ohnishi, O. (1982). Population genetics of cultivated buckwheat, *Fagopyrum esculentum* Moench, I. Frequency of chlorophyll-deficient mutants in Japanese populations. *Japanese Journal of Genetics* **57**, 623–639.
Rose, M. R. (1982). Antagonistic pleiotropy, dominance, and genetic variation. *Heredity* **48**, 63–78.
Saccheri, I. J., Brakefield, P. M. & Nichols, R. A. (1996). Severe inbreeding depression and rapid fitness rebound in the butterfly *Bicyclus anynana* (Satyridae). *Evolution* **50**, 2000–2013.
Schmitt, J. & Ehrhardt, D. W. (1987). A test of the sib-competition hypothesis for outcrossing advantage in *Impatiens capensis*. *Evolution* **41**, 579–590.
Schuler, J. F. (1954). Natural mutations in inbred lines of maize and their heterotic effect. I. Comparison of parent, mutant and their F1 hybrid in a highly inbred background. *Genetics* **39**, 908–922.
Schuler, J. F. & Sprague, G. F. (1955). Natural mutations in inbred lines of maize and their heterotic effect. II. Comparison of mother line versus mutant when outcrossed to related inbreds. *Genetics* **41**, 281–291.
Shabalina, S. A., Yampolsky, L. Y. & Kondrashov, A. S. (1997). Rapid decline of fitness in panmictic populations of *Drosophila melanogaster* maintained under relaxed selection. *Proceedings of the National Academy of Sciences of the USA* **94**, 13034–13039.
Simmons, M. J. & Crow, J. F. (1977). Mutations affecting fitness in *Drosophila* populations. *Annual Review of Genetics* **11**, 49–78.
Smouse, P. E. (1986). The fitness consequences of multiple-locus heterozygosity under the multiplicative overdominance and inbreeding depression models. *Evolution* **40**, 946–957.
Strauss, S. H. (1986). Heterosis at allozyme loci under inbreeding and crossbreeding in *Pinus attenuata*. *Genetics* **113**, 115–134.
Strauss, S. H. & Libby, W. J. (1987). Allozyme heterozygosity in radiata pine is poorly explained by overdominance. *American Naturalist* **130**, 879–890.
Stuber, C. W., Lincoln, S. E., Wolff, D. W., Helentjaris, T. & Lander, E. S. (1992). Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics* **132**, 823–839.
Sved, J. A. (1971). An estimate of heterosis in *Drosophila melanogaster*. *Genetical Research* **18**, 97–105.
Sved, J. A. (1975). Fitness of third chromosome homozygotes in *Drosophila melanogaster*. *Genetical Research* **25**, 197–200.
Sved, J. A. & Wilton, A. N. (1989). Inbreeding depression and the maintenance of deleterious genes by mutation: model of a *Drosophila* chromosome. *Genetical Research* **53**, 119–128.

Thornhill, N. W. (1993). *The Natural History of Inbreeding and Outbreeding*. Chicago, IL: University of Chicago Press.

Tracey, M. L. & Ayala, F. J. (1974). Genetic load in natural populations: is it compatible with the hypothesis that many polymorphisms are maintained by natural selection? *Genetics* **77**, 569–589.

Uyenoyama, M. K., Holsinger, K. E. & Waller, D. M. (1993). Ecological and genetic models of the evolution of self-fertilization. *Oxford Surveys in Evolutionary Biology* **9**, 327–381.

Wang, J., Hill, W. G., Charlesworth, D. & Charlesworth, B. (1999). Dynamics of inbreeding depression due to deleterious mutations in small populations. I. Mutation parameters and inbreeding rate. *Genetical Research*, **74**: 165–178.

Watanabe, T. K. & Ohnishi, S. (1975). Genes affecting productivity in natural populations of *Drosophila melanogaster*. *Genetics* **80**, 807–819.

Willis, J. H. (1992). Genetic analysis of inbreeding depression caused by chlorophyll-deficient lethals in *Mimulus guttatus*. *Heredity* **69**, 562–572.

Willis, J. H. (1993). Effects of different levels of inbreeding on fitness components in *Mimulus guttatus*. *Evolution* **47**, 864–876.

Willis, J. H. (2000). The contribution of male sterility mutations to inbreeding depression in *Minulus guttatus*. *Heredity*, in press.

Willis, J. H. (2000). The role of genes of large effect on inbreeding depression in *Mimulus guttatus*. *Evolution*, in press.

Wilton, A. N. & Sved, J. A. (1979). X-chromosomal heterosis in *Drosophila melanogaster*. *Genetical Research* **34**, 303–315.

Wilton, A. N., Joseph, M. G. & Sved, J. A. (1989). Can chromosomal heterosis be explained by deleterious recessive genes? Negative results from a chromosomal population test. *Genetical Research* **54**, 129–140.

Wright, S. (1977). *Evolution and the Genetics of Populations*, vol. 3, *Experimental Results and Evolutionary Deductions*. Chicago, IL: University of Chicago Press.

Zamir, D. & Tadmor, Y. (1986). Unequal segregation of nuclear genes in plants. *Botanical Gazette* **147**, 355–358.

Zouros, E. & Foltz, D. W. (1984). Possible explanations of heterozygote deficiency in bivalve molluscs. *Malacology* **25**, 583–591.

Zouros, E., Singh, S. M. & Miles, H. E. (1980). Growth rate in oysters: an overdominant phenotype and its possible explanations. *Evolution* **34**, 856–867.