THE ROYAL SOCIETY

Published online 20 May 2003

Recent approaches into the genetic basis of inbreeding depression in plants

David E. Carr[1*] and Michele R. Dudash[2]

[1]*Blandy Experimental Farm, University of Virginia, 400 Blandy Farm Lane, Boyce, VA 22620, USA*
[2]*Department of Biology, University of Maryland, College Park, MD 20742-5815, USA* (*mdudash@umd.edu*)

Predictions for the evolution of mating systems and genetic load vary, depending on the genetic basis of inbreeding depression (dominance versus overdominance, epistasis and the relative frequencies of genes of large and small effect). A distinction between the dominance and overdominance hypotheses is that deleterious recessive mutations should be purged in inbreeding populations. Comparative studies of populations differing in their level of inbreeding and experimental approaches that allow selection among inbred lines support this prediction. More direct biometric approaches provide strong support for the importance of partly recessive deleterious alleles. Investigators using molecular markers to study quantitative trait loci (QTL) often find support for overdominance, though pseudo-overdominance (deleterious alleles linked in repulsion) may bias this perception. QTL and biometric studies of inbred lines often find evidence for epistasis, which may also contribute to the perception of overdominance, though this may be because of the divergent lines initially crossed in QTL studies. Studies of marker segregation distortion commonly uncover genes of major effect on viability, but these have only minor contributions to inbreeding depression. Although considerable progress has been made in understanding the genetic basis of inbreeding depression, we feel that all three aspects merit more study in natural plant populations.

Keywords: dominance; epistasis; inbreeding; mating systems; overdominance; recessive mutations

1. INTRODUCTION

The extent of gene flow in plants will be determined in part by the degree to which they outcross. Because most plants can function as both male and female, self-fertilization is often possible, and *ca*. 20% of plants surveyed have selfing rates in excess of 80% (Barrett & Eckert 1990; Vogler & Kalisz 2001). Selfing offers several obvious ecological advantages such as pollination assurance and colonizing ability and is one of the most widespread evolutionary phenomena in plants (Stebbins 1974). Fisher (1941) noted that alleles that increase the selfing rate without impacting the ability of the plant to serve as a pollen donor can enjoy a transmission advantage that will lead to their rapid spread, even without the ecological conditions that might otherwise promote selfing, yet mechanisms that promote outcrossing abound. Opposing this transmission advantage is the reduction in offspring quality (inbreeding depression) that often accompanies self-fertilization and other forms of inbreeding (Charlesworth & Charlesworth 1987). Predictions about the evolutionary stability of outcrossing and selfing vary, depending on the genetic basis of inbreeding depression (Uyenoyama *et al.* 1993).

In this review, we examine several recent attempts to identify the genetic basis of inbreeding depression in plants, concentrating primarily on natural populations. We include three aspects of the genetic basis that have been identified as essential to predicting the evolution of mating systems and the genetic load of plant populations:

(i) the type of allelic interaction involved (dominance or overdominance);
(ii) the contribution of epistasis; and
(iii) the number of loci and the distribution of their effects.

We conclude that the evidence points towards an important role of deleterious recessive alleles and the polygenic nature of inbreeding depression. Recent marker-aided analyses (primarily of crops) have repeatedly suggested contributions of overdominant loci, however. Although epistasis historically has been difficult to detect, new marker-assisted techniques have suggested that its importance in the inbreeding process may be underappreciated.

The fundamental genetic change that inbreeding produces, a loss of heterozygosity, was well understood early in the development of the field of genetics (Wright 1921). Selfing, the most extreme form of inbreeding, reduces heterozygosity by 50% each generation. The decrease in heterozygosity typically results in phenotypic changes that we call inbreeding depression, the genetic basis of which has been debated for almost a century. The two competing (though not necessarily mutually exclusive) explanations are commonly referred to as the dominance (or part dominance) and overdominance hypotheses (Charlesworth & Charlesworth 1987).

*Author for correspondence (dec5z@virginia.edu).

One contribution of 21 to a Discussion Meeting Issue 'Mechanisms regulating gene flow in flowering plants'.

The dominance hypothesis states that inbreeding depression results from the increased homozygosity of recessive or partly recessive deleterious alleles, the effects of which are masked or partly masked by dominant alleles in more heterozygous, outbred offspring (Davenport 1908). The overdominance hypothesis states that heterozygotes at a given locus have an inherent advantage over homozygotes and that the loss of heterozygosity in inbred progeny results in inbreeding depression (East 1908; Shull 1908). Although the dominance and overdominance hypotheses predict the same qualitative outcome of inbreeding from one generation to the next, they differ greatly in the expectations for evolution within populations.

Under the dominance hypothesis, deleterious alleles are expected to be maintained in a random-mating population at a selection–mutation balance that would depend on the rate of mutation, the effect of the alleles and the degree to which alleles are expressed in heterozygotes. The lower the mutation rate, the more severe the effect of the allele, and the more the deleterious effect is expressed in heterozygotes, the lower will be the expected frequency of the allele at equilibrium. The existence of these alleles in a population is commonly referred to as the 'mutational load'. Several models of mating system evolution predict that if inbreeding depression is due to mutational load, two stable equilibria exist. If inbreeding depression (δ), measured as one minus the ratio of the fitness (w) of self and outcross progeny ($\delta = 1 - w_{\mathrm{self}}/w_{\mathrm{outcross}}$), is greater than 50%, outcrossing will be stable; if $\delta < 50\%$ then selfing will be stable (Lande & Schemske 1985; D. Charlesworth *et al.* 1990).

Genetic variation at an overdominant locus is expected to be maintained by balancing selection. The high fitness of heterozygous genotypes favours the persistence of an allelic polymorphism in the population. Because heterozygotes do not breed true, half of their offspring will be homozygous at any given locus, and the production of these low fitness homozygous genotypes in a population is commonly referred to as a 'segregation load.' As is the case for mutational load, inbreeding depression produced from the segregation load in excess of $\delta = 50\%$ will prevent the spread of an allele that increases the selfing rate and will maintain complete outcrossing (Ziehe & Roberds 1989; Charlesworth & Charlesworth 1990). Predictions assuming a segregation load from overdominance differ from those assuming a mutational load from recessive mutations in that intermediate selfing rates can be evolutionarily stable even if inbreeding depression is less than 50% (Holsinger 1988; Charlesworth & Charlesworth 1990).

One of the difficulties that has plagued even the best efforts to distinguish between these two genetic mechanisms is that two closely linked loci (A and B) segregating deleterious alleles in repulsion phase (i.e. the deleterious alleles of the two loci are borne on opposite homologous chromosomes) can produce an effect that appears in a genetic analysis as overdominance at a single locus. This phenomenon, often referred to as 'pseudo-overdominance', occurs because the dominant allele on one homologue complements the deleterious allele on the other. Individuals heterozygous for this linkage group will appear to have the highest fitness because they bear a dominant allele at both locus A and B. In homozygotes, deleterious recessive alleles at one of the two loci will always be expressed. As we will see, this has made definitive support for overdominance difficult to establish.

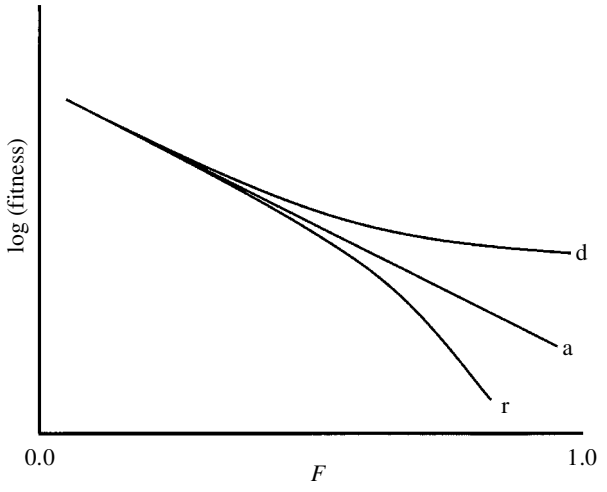

Figure 1. Multiplicative gene action for a quantitative trait exhibiting inbreeding depression results in an expected log-linear decline (a) with increasing genome homozygosity (F). Epistasis can cause a curvilinear response, either concave up (diminishing epistasis, d) or concave down (reinforcing epistasis, r).

Early surveys of outcrossing rates supported the prediction of the dominance hypothesis for a bimodal distribution (Schemske & Lande 1985; Barrett & Eckert 1990), but a reinterpretation of these data found that the prediction of a bimodal distribution is supported only in wind-pollinated species, with almost half of insect-pollinated species having outcrossing rates between 20% and 80% (Vogler & Kalisz 2001). Although the overdominance hypothesis predicts potentially stable intermediate outcrossing rates, it is unknown whether the intermediate outcrossing rates observed in many natural populations are in fact stable, whether they are transient states of populations evolving to one extreme or the other, or whether these models are too simplistic to make accurate predictions about the evolution of the selfing rate (Lloyd 1992; Uyenoyama *et al.* 1993; Holsinger 1993, 1996; Schoen *et al.* 1996).

There are two other aspects to the genetic basis of inbreeding depression that influence the prediction of how mating systems and genetic load will evolve in a population. First, the interactive effects among loci (epistasis) will affect how fitness changes with increasing homozygosity (Crow & Kimura 1970). With no epistasis, fitness should decline log-linearly as a population becomes more inbred. It may be the case, however, that the individual deleterious effect of homozygosity at a given locus becomes greater with the increasing homozygosity of the genome (reinforcing epistasis). Alternatively, it may be that the individual effects become weaker (diminishing epistasis) with increasing genomic homozygosity (figure 1). If either type of epistasis exists, the effect of any mutation is not independent of its genetic background, and a change in the rank order of allelic effects can even generate apparent overdominance (Goodnight 1999). Reinforcing epistasis may favour an intermediate outcross-

ing rate (as predicted with the overdominance model; Charlesworth & Charlesworth 1990) because it will cause inbreeding depression to increase with increasing homozygosity (Charlesworth *et al.* 1991).

Finally, the number of loci and the distribution of their effects will also influence how mating systems and genetic load will evolve in a population (Barrett & Charlesworth 1991; Husband & Schemske 1996; Willis 1999*a*). Recessive mutations of major effect can be more easily acted upon by natural selection, making their removal from an inbreeding population highly probable. Mutations of small effect are likely to be fixed by drift in an inbreeding population. A major effect of a single overdominant locus can also have an unexpected effect on mating system evolution, enabling evolutionarily stable mixed mating (Uyenoyama & Waller 1991).

2. DOMINANCE AND OVERDOMINANCE

(a) *Purging*

An important prediction of the dominance hypothesis is that inbreeding and selection should work together to reduce a population's mutational load (B. Charlesworth *et al.* 1990). Inbreeding increases the homozygosity of deleterious recessive alleles and thus increases their phenotypic expression, making selection more efficient at removing or 'purging' the alleles from the inbred line and the population. Consequently, populations that have had a history of inbreeding are expected to have a lower mutational load, lower inbreeding depression and higher mean fitness than their ancestral populations. By contrast, the overdominance hypothesis predicts that inbreeding depression will increase as a population becomes more inbred because of the loss of heterozygote advantage at many loci (Charlesworth & Charlesworth 1990). The polymorphisms that had been maintained by balancing selection will probably be lost eventually owing to genetic drift. This will lower the inbreeding depression in the population but at a cost of lowering the mean fitness of the inbred population relative to the base population from which it was derived.

Evidence of a purging effect via inbreeding has been sought in comparisons of populations or closely related species that differ significantly in their outcrossing rates. In a survey of studies that examined 54 species of plants comprising 79 populations, Husband & Schemske (1996) indeed found a significant negative correlation between selfing rate and inbreeding depression, with differences between selfing and outcrossing species particularly pronounced in early life-history traits. However, inbreeding depression in early life-history traits may be uncorrelated to inbreeding depression in later life-history traits (Carr & Dudash 1995), and substantial inbreeding depression can still be found in highly selfing species, especially in these later life-history traits (B. Charlesworth *et al.* 1990; Husband & Schemske 1996). Highly selfing species may, in fact, accumulate deleterious mutation owing to 'Muller's ratchet' (Kondrashov 1994; Wang *et al.* 1999*b*; Bustamante *et al.* 2002). Interpreting a lack of inbreeding depression in highly selfing populations is complicated by the fact that the fixation of alleles through drift produces the same effect as the selective removal of deleterious alleles; without genetic variation, inbreeding depression will always be zero. In their review, Byers & Waller (1999) concluded that the evidence for purging from these types of comparison is limited at best, with only 14 out of 34 plant studies that made comparisons among populations or species finding evidence of reduced inbreeding depression in populations with higher selfing rates.

It is, in part, because of the difficulties involved in interpreting comparative studies, that evidence for purging has also been sought by examining changes in fitness and inbreeding depression in primarily outcrossing species that are experimentally inbred over several generations. Assuming multiplicative gene action and no selection, the mean value of a trait should decline log-linearly as a population becomes progressively more inbred under both the dominance and overdominance hypotheses. For fitness traits, however, selective purging of deleterious alleles from the inbreeding population during each generation should result in an attenuation of the fitness decline. Furthermore, if pairs of inbred lines derived from the same base population are crossed, their progeny are expected to exceed the base population mean because partly recessive alleles that had expressed deleterious effects (at least to some degree) in the base population will have been removed from the inbred lines. If inbreeding depression is due to overdominance, crosses among inbred lines may return fitness to the level of the base population but no higher.

The greenhouse study of Barrett & Charlesworth (1991) of a highly outbreeding population of *Eichhornia paniculata* (water hyacinth) was the first, to our knowledge, to demonstrate purging in plants drawn from natural populations. After five consecutive generations of enforced selfing, inbred lines were crossed. Although the performance of inbred plants did not improve over the course of the enforced inbreeding, flower production by plants derived from the crosses between inbred lines was much greater than in the base population, a result entirely consistent with the dominance hypothesis and incompatible with the overdominance hypothesis.

Purging was not observed, however, in our greenhouse studies of two populations of the monkey flower, *Mimulus guttatus* (Dudash *et al.* 1997; Carr & Dudash 1997). As in the *Eichhornia* study, we inbred this mixed-mating species for five generations, but we did not observe an increase in the mean performance of crosses between inbred lines at any stage of the inbreeding process. This was true for later life-history traits (flower production and total above-ground biomass) and for fertility traits (pollen number, pollen viability and ovule number) that were likely under selection during the course of the breeding programme. Instead, fitness traits declined continuously in the inbreeding populations, and outcrossing brought these traits up no higher than in the original populations. We interpreted the response as indicating the random fixation of deleterious alleles within our inbred lines, but we could not exclude overdominance based on these data alone.

Although our studies on *M. guttatus* showed no evidence of selective purging of alleles from either of our populations, we lost up to 50% of families during the inbreeding process, and families within both populations varied significantly in their response to inbreeding, with some lines showing patterns consistent with purging

(Dudash *et al.* 1997; Carr & Dudash 1997). We suggested, as had Falconer (1981) based on inbred lines of mice, that selection among lines would be more effective in purging mutational load than selection within lines.

The effect of this among-line selection was observed in a greenhouse study of 1200 inbred lines of *M. guttatus* by Willis (1999*a*). Only 335 lines survived through the full five generations of selfing, suggesting that many of the deleterious alleles in the base population could have been eliminated with the failed lines. After five generations of inbreeding, lines were crossed to form an F_1 outbred population, and from this F_1 population, inbred and outbred F_2 progeny were produced. The mean performance of these inbred and outbred F_2 progeny exceeded the mean for inbred and outbred plants, respectively, from the ancestral population for nearly all fitness traits. Mean performance of inbred F_2 progeny was actually equivalent to the mean performance of outbred plants derived from the ancestral population for all cumulative measures of fitness. These results are consistent with the dominance hypothesis and suggest purging. However, the level of inbreeding depression (δ) measured in these F_2 plants was essentially unchanged relative to the ancestral population, counter to expectations.

Willis (1999*a*) explained his seemingly conflicting results by hypothesizing that during the inbreeding programme, plants became adapted to the greenhouse environment but retained the mutational load of the ancestral population (with the exception of most lethals and steriles). This would seem to imply that adaptation was due to additive effects almost exclusively, but we feel that there is no definitive evidence to discount the role of selection against partly recessive deleterious alleles in the improved performance of the F_2 inbred and outbred plants. We offer an alternative hypothesis to explain the failure of the purging process to reduce inbreeding depression. It seems likely that during the inbreeding process selection would act on multilocus epistatic genotypes (see § 3). Recombination in the F_2 generation would break up positive epistatic interactions, reducing the mean performance of the self plants and probably resulting in higher inbreeding depression than would be expected under a purely additive model (Lynch & Walsh 1998). A role of overdominance cannot be discounted either. Regardless, it is clear from this result (Willis 1999*a*) that although purging may be capable of removing some deleterious alleles from an inbreeding population, substantial inbreeding depression can remain.

Byers & Waller (1999) reviewed 13 plant studies of sequential inbreeding (including the *Eichhornia* and *Mimulus* studies described already) and reported only five cases of purging at either the species, population or lineage level. However, most of these studies followed plants through only two generations of selfing, and only two studies, both of which found some evidence consistent with the purging hypothesis (McCall *et al.* 1994; Willis 1999*a*), appear to have allowed selection among lineages. The meta-analysis of Crnokrak & Barrett (2002) of both plant and animal studies of purging found strong general support for the phenomenon in plants when the performance of outbred plants derived by crossing highly inbred lines was compared with the ancestral outbred population. Crnokrak & Barrett (2002) pointed out, however, that conclusions drawn in studies of purging could vary, depending on how one evaluated purging.

(b) *Biometric approaches*

Biometric approaches to understanding the types of allelic interaction involved in inbreeding depression and its converse, heterosis, have been developed and applied to crops, particularly maize (Simmonds 1981; Hallauer & Miranda 1985). Only recently have these approaches been adopted for investigating inbreeding depression in natural populations. These techniques do not describe the interaction of alleles at any given locus but rather estimate the average type of allelic interaction across all loci affecting a trait. The sum across loci for this average is weighted by the strength of their effects on the trait, such that loci of greater effect are weighted more heavily.

Mukai *et al.* (1972) developed a regression technique for estimating average dominance ($\bar{h}$) using inbred lines. Inbred lines are crossed, and fitness traits of F_1 heterozygotes are regressed on the sum of the fitness of their parental inbred lines. The slope of this regression is equivalent to the average dominance. Purely additive effects are indicated by $\bar{h} = 0.5$, and complete dominance is indicated by $\bar{h} = 0$. Values between 0 and 0.5 indicate part dominance and values below 0 indicate overdominance. Under the likely scenario of a contribution of both dominance and overdominance to inbreeding depression, however, this technique is strongly biased in favour of dominance (Deng 1998; Lynch & Walsh 1998).

Johnston & Schoen (1995) applied this technique to two populations each of two highly selfing species of *Amsinckia*. Inbreeding depression was found to be low in both species, with estimates of δ for total fitness (the number of autonomously set seed per seed planted) of 11% and 17% for the two populations of *A. gloriosa* and 14% and 10% for the two populations of *A. spectabilis*. Estimates of average dominance for all traits in *A. gloriosa* and for flower production in *A. spectabilis* indicate that partly recessive alleles are responsible for inbreeding depression (table 1). However, in the Zmudowski State Beach population of *A. spectabilis*, neither recessive mutations nor overdominance could be ruled out for the only trait showing significant inbreeding depression, 'total fitness'.

The most powerful biometric approach for estimating average dominance is the North Carolina 3 (figure 2; Comstock & Robinson 1952; Kearsey 1980). Average dominance ($\bar{a}$) is measured as the square-root of the ratio of the F_2 sire × inbred line interactive variance component (non-additive genetic variation) to the variance among the F_2 sires (additive genetic variation). Purely additive allele action is indicated by $\bar{a} = 0$, and complete dominance is indicated by $\bar{a} = 1.0$. Partial dominance is indicated by values between 0.0 and 1.0, and overdominance is indicated by values of $a > 1.0$. Note that both $\bar{a}$ and $\bar{h}$ are proportional to the deviation of the heterozygote from the midpoint of the corresponding homozygotes, but $\bar{h}$ is inversely proportional, and $\bar{a}$ is directly proportional. They also differ in scale by a factor of two, but $\bar{a}$ can be converted to $\bar{h}$ for comparison as $\bar{h} = (1 - \bar{a})/2$.

We applied this to two populations of the mixed-mating *M. guttatus* and the highly selfing *M. micranthus* (Dudash & Carr 1998). Many studies have demonstrated inbreeding depression in *M. guttatus* for a wide range of

Table 1. Estimates of average dominance ($\bar{h}$) and 95% confidence limits (95% CL) for four *Amsinckia* populations (after Johnston & Schoen 1995).
(Estimates were derived from the regression technique of Mukai *et al.* (1972). Purely additive effects are indicated by $\bar{h} = 0.5$. Values of $\bar{h}$ between 0 and 0.5 indicate partly recessive deleterious alleles. Values of $\bar{h} < 0$ indicate overdominance. Total fitness was calculated as the total autonomous seed set per seed planted.)

	Amsinckia gloriosa				*Amsinckia spectabilis*			
	Paloma Creek Canyon		New Iridia		Alisal Slough		Zmudowski State Beach	
trait	$\bar{h}$	95% CL	$\bar{h}$	95% CL	$\bar{h}$	95% CL	$\bar{h}$	95% CL
survival to flowering	0.32[a]	0.25, 0.40	0.27[a]	0.12, 0.47	0.41	0.27, 0.55	0.45	0.25, 0.67
flowers production	0.23[a]	0.14, 0.31	0.32	0.17, 0.50	0.32	0.12, 0.51	0.31	0.14, 0.53
autonomous seed set	0.25[a]	0.16, 0.34	0.26	0.11, 0.57	−0.20[c]	−0.59, −0.05	0.08[b]	−0.29, 0.28
total fitness	0.28[a]	0.15, 0.43	0.35[a]	0.26, 0.47	0.14	−0.32, 0.59	0.07[b]	−0.25, 0.37

[a] Estimates significantly less than 0.5 and significantly greater than 0.
[b] Estimates significantly less than 0.5 but not significantly different from 0.
[c] Estimates significantly less than 0.

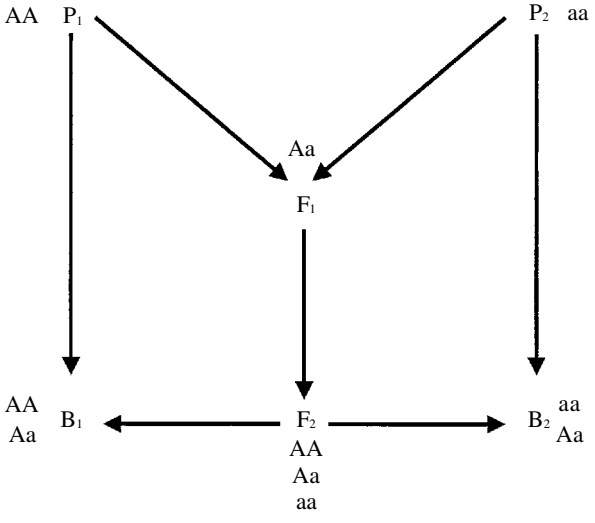

Figure 2. The North Carolina 3 breeding design. Inbred lines are crossed to form genetically uniform F_1 hybrids. F_1 hybrids are selfed to form segregating F_2 progeny. F_2 sires are used in backcrosses to the parental inbred lines.

fitness components (Willis 1993*a*,*b*; Latta & Ritland 1994; Carr & Dudash 1995). In both of our *M. guttatus* populations our estimates of average dominance indicated that inbreeding depression for flower production, biomass and ovule production was due to partly recessive deleterious alleles (table 2). In both *M. guttatus* populations, inbreeding depression for pollen viability was due to nearly completely recessive deleterious alleles. The two populations differed in the type of dominance involved with inbreeding depression for pollen production. In one population (*S*), deleterious alleles were estimated to be weakly recessive. In population *T* our estimates indicated significant overdominance, although pseudo-overdominance could not be completely ruled out in this case. The crosses between inbred lines at the start of this breeding design generated complete linkage disequilibrium in the F_1 generation, and this disequilibrium was only partly eroded by recombination in the F_2. Performing backcrosses after the F_3 or later generations would allow for more recombination among linked loci and could enable this pseudo-overdominance to be distinguished from true overdominance, but we have not done these crosses.

In the highly selfing *M. micranthus* we found inbreeding depression for biomass and flower production, and our dominance estimates indicated that this was due to partly recessive deleterious alleles and not to a segregation load that selection was unable to eliminate (table 2). Interestingly our *M. micranthus* estimates indicated alleles that are more nearly additive than the estimates for the same fitness components in *M. guttatus*. This is what would be expected after purging in an inbreeding population; inbreeding would have its most pronounced effects on the selection–mutation balance of more highly recessive alleles but would have less effect on the equilibrium of more additive alleles because these have expression even in outcrossing populations (D. Charlesworth *et al.* 1990). We did not see inbreeding depression for pollen or ovule production in *M. micranthus* (Carr & Dudash 1996). This was consistent with the hypothesis that natural selection removes deleterious recessive alleles from inbreeding populations, but we could not test this with the North Carolina 3 design directly.

Our estimate of average dominance for flower production in the selfing *M. micranthus* was quite similar to the corresponding estimates from the highly selfing species of *Amsinckia* (table 1; Johnston & Schoen 1995), with our estimate falling comfortably within their 95% confidence intervals. The estimates of dominance for flower production in *Amsinckia* were also more additive than the corresponding estimates in mixed-mating *M. guttatus*, with all *M. guttatus* estimates falling below the *Amsinckia* confidence intervals (cf. tables 1 and 2). Again, the more additive alleles in these selfing species are as predicted by the dominance hypothesis (D. Charlesworth *et al.* 1990).

Willis (1999*b*) also employed the Mukai *et al.* (1972) regression technique to estimate average dominance ($\bar{h}$) in a study of 184 highly inbred lines of *M. guttatus* derived from a natural population at Iron Mountain, OR, USA. These estimates were similar to our own for three of the four traits where direct comparisons can be made, with almost all of our estimates falling within his reported 95% confidence intervals (table 1). For the notable exception,

Table 2. Estimates of average dominance for three populations of the mixed-mating *Mimulus guttatus* and one population of the highly selfing *M. micranthus*.
(Average dominance ($\bar{a}$) for *M. guttatus* populations S and T and for *M. micranthus* are derived from a North Carolina 3 breeding design (Dudash & Carr 1998). Values of $\bar{a}$ not significantly different from 1.0 indicate completely recessive alleles. Values of $\bar{a}$ significantly less than 1.0, but significantly greater than 0.0 (pure additivity) indicate partly recessive alleles. Values of $\bar{a}$ significantly greater than 1.0 ($^{a}p < 0.05$) indicate overdominance. Estimates of average dominance ($\bar{h}$) and 95% confidence limits (95% CL) from the Iron Mountain populations are based on the Mukai *et al.* (1972) regression technique and are taken from Willis (1999*b*). Values of $\bar{h}$ significantly greater than 0.0, but less than 0.5 (pure additivity) indicate partly recessive alleles. Estimates of $\bar{a}$ from Dudash & Carr (1998) were converted to $\bar{h}$ ($\bar{h} = (1 - \bar{a})/2$) for comparison purposes.)

	average dominance ($\bar{a}$)		average dominance ($\bar{h}$)		average dominance ($\bar{h}$)	95% CL	average dominance ($\bar{a}$)	average dominance ($\bar{h}$)
trait	S	T	S	T	Iron Mountain		*Mimulus micranthus*	
germination	—	—	—	—	0.213***	0.100, 0.326	—	—
flowers	0.778*	0.741*	0.111	0.130	0.057	–0.058, 0.172	0.436***	0.282
biomass	0.747*	0.692**	0.127	0.154	—	—	0.577***	0.212
ovule number	0.613***	0.601***	0.194	0.120	0.179***	0.101, 0.257	—	—
pollen viability (%)	0.972	0.931	0.014	0.035	0.063*	0.015, 0.111	—	—
pollen number	0.174***	1.321[a]	0.413	−0.161	0.122***	0.064, 0.180	—	—
viable pollen/ flower	—	—	—	—	0.087***	0.039, 0.135	—	—

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

pollen production, the genetic load in our population *S* was more nearly additive, and our population *T* showed evidence of overdominance. As with our estimates for *M. guttatus*, the dominance estimates from this outcrossing Iron Mountain population were also all more highly recessive than the corresponding estimates in the highly selfing *M. micranthus* and in the highly selfing *Amsinckia* species.

(c) *Marker-aided approaches*

Relationships between multilocus heterozygosity of genetic markers and fitness have generated debate about the relative importance of overdominance and dominance to genetic load, although these relationships are weak at best and have contributed little to the resolution of the problem (Britten 1996; David 1998; Slate & Pemberton 2002). Smouse (1986) derived a model based on the assumption that the correlation between fitness and heterozygosity at marker loci (in particular, allozymes) was due to multiplicative overdominance. The model quantified multilocus genotypes as to the degree to which they deviated from the optimal, entirely heterozygous genotype (the genotype's 'adaptive distance'), with the less frequent of the two homozygotes at each locus regarded as being more distant from the optimum. If adaptive distance accounted for a significantly greater proportion of the variance of log-transformed fitness than a simpler model using only the number of heterozygous loci, then this was interpreted as support for overdominance. Bush *et al.* (1987) used this approach in concluding that overdominance better accounted for variation in growth rate of *Pinus rigida* (pitch pine). Houle (1994) demonstrated that in the case of identity disequilibrium (generated by inbreeding; Weir & Cockerham 1973) or linkage disequilibrium, the adaptive distance model fits as well under the assumption of dominance as it does with overdominance. The adaptive distance model cannot, therefore, unambiguously distinguish between the two models of inbreeding depression. David (1997) has extended the models of Houle (1994) to generate three tests that might be able to provide some resolution, although to our knowledge, these tests have yet to be applied to plant populations.

The use of molecular markers in concert with breeding designs has greater power to distinguish between models of inbreeding depression than genetic surveys of heterozygosity in natural populations. Two distinct approaches, each yielding somewhat different information, have been taken: the identification of 'QTL' and the detection of genes affecting viability by testing for marker segregation distortion. Both these techniques use variable molecular markers (though morphological markers with simple Mendelian inheritance could also be used) such as allozyme loci, RFLPs, AFLPs, RAPD and simple sequence repeats (or microsatellites) to infer linkage to loci contributing to inbreeding depression or heterosis. We shall first describe the use of molecular markers to infer loci that affect quantitative traits.

The advent of molecular techniques for studying genes affecting quantitative traits holds great promise for answering detailed questions about the genetic basis of inbreeding depression. Crossing between lines differing in both phenotype and markers and using these hybrid progeny to produce segregating generations (e.g. F_2 or backcross) will produce marker genotypes that co-segregate with linked QTL. The linkage between markers and QTL can be detected by differences in mean phenotype

among marker genotypes. The degree of linkage disequilibrium between homozygous marker genotypes and the QTL will be proportional to the map distance between the QTL and the marker and the effect of the QTL on the trait. The difference between the phenotypes of the heterozygous marker genotype and the homozygous genotypes will be proportional to the dominance deviation and the map distance (Mackay 2001). Knowledge of the map location of the marker allows for localization of QTL within the genome. As with the biometric approaches to average dominance, inference drawn from QTL analyses will be biased toward those loci producing the greatest phenotypic effects, but QTL analysis has the advantage of allowing dominance estimates for a single locus or multiple closely linked loci within a marker interval.

The power of a QTL analysis will depend on the degree to which the molecular markers saturate the genome (the more powerful studies average a marker approximately every 10 cM) and the divergence of the lines with respect to the phenotypic characters of interest. For this reason, most QTL analyses of heterosis and inbreeding depression have been conducted on crops where crosses between highly divergent lines are often of interest and high density maps can be constructed owing to economic and social incentives to produce such maps. Using these techniques to make inferences about the genetic basis of inbreeding depression in natural populations may be hindered somewhat because

(i) genetic markers and phenotypic diversity within a single population may be more limited; and
(ii) crosses between more divergent populations may be of questionable utility for understanding the evolution of mating systems and genetic load within populations.

We shall, therefore, first discuss the findings of several crop studies.

Using 76 RFLP and allozyme markers and a North Carolina 3 breeding design taken to the F_3 generation, Stuber *et al.* (1992) inferred the genetic basis of heterosis for grain yield and six other quantitative traits (ear and plant height, ear leaf area, days to tassel, grain moisture, ears per plant and grain yield) in a cross between two elite inbred lines of maize (*Zea mays*). With a single exception, every time a QTL for grain yield was detected, plants that were heterozygous for the marker had the highest yield. The QTL for yield also tended to occur at the same map location for both backcrosses. These patterns lead to the conclusions that heterosis in this case is due to overdominance (table 3). As in our biometric analysis of *M. guttatus* (Dudash & Carr 1998), pseudo-overdominance cannot be ruled out completely, although the use of F_3 sires in the maize study increased recombination over the F_2. Allowing too much recombination, however, can destroy the linkage disequilibrium between markers and QTL, defeating the purpose of the analysis.

Cockerham & Zeng (1996) recently extended the North Carolina 3 analysis of variance (Comstock & Robinson 1952) to include linkage, pairwise epistasis (see § 3) and orthogonal contrasts for single genetic markers. They then applied this new model to the maize data from Stuber *et al.* (1992). The average degree of dominance ($\bar{a}$, as in Comstock & Robinson 1952) for yield was 1.96, indicating overdominance. Estimates for six other traits were all significantly less than 1.0, indicating partly recessive alleles. Consideration of the marker contrasts produced results that were in keeping with the average dominance estimated from the analysis of variance. Analysis of the epistatic effects (see § 3) indicated that there were likely multiple QTL per chromosome, many of which did not show up individually as significant QTL in the analysis. Cockerham & Zeng (1996) indicated that this could easily result in pseudo-overdominance. The striking difference between the estimated gene actions for yield (overdominance) relative to the other six traits (partly recessive alleles) may be due to a greater potential for pseudo-overdominance because of a larger number of loci contributing to yield, which itself is a product of several of the other traits. A major QTL on chromosome 5 that had originally suggested overdominance has been more finely mapped, and indeed the region was found to host two QTL with deleterious recessive mutations linked in repulsion (Graham *et al.* 1997). Further fine-scale analyses of other QTL are needed to discover the full extent of pseudo-overdominance in maize.

The genetic basis of inbreeding depression in rice (*Oryza sativa*), a highly self-pollinating species, potentially offers an interesting contrast to the more outcrossing maize. Despite a highly selfing mating system, F_1 hybrids between two inbred lines showed strong heterosis for biomass (as much as a 101% increase over the midparent expectation) and grain yield (a 120% increase; Li *et al.* 2001). RILs descended from these F_1 plants after 10 generations of selfing showed strong inbreeding depression (ranging from 40 to 47% for the grain yield), and backcrosses and testcrosses using the RIL showed significant heterosis. Using 182 RFLP markers, Li *et al.* (2001) concluded that overdominance was the cause of heterosis for grain yield and biomass in *ca.* 90% of detectable QTL, and Luo *et al.* (2001) found that overdominance was responsible for heterosis in most components of grain yield (table 3). Both sets of authors provided strong arguments against pseudo-overdominance. This was at odds with an earlier analysis that strongly favoured the dominance hypothesis (Xiao *et al.* 1995), and Li *et al.* (2001) attributed this to the earlier estimation technique's inability to incorporate epistasis (see § 3).

A second marker-aided approach tests for segregation distortion of the marker alleles. The distortion is presumably produced by loci segregating alleles affecting viability. Marker genotypes linked to loci that reduce viability will be underrepresented in sets of progeny. The simplest application is to self a typically outbred plant and test for segregation distortion in these inbred progeny. In plants with highly selfing mating systems, crosses are typically made between inbred lines and the F_1 progeny are selfed to produce segregating F_2 progeny.

Fu & Ritland (1994*a*) developed a graphical technique for examining segregation distortion of genetic markers, plotting observed segregation ratios against the frequency of the less common homozygote (figure 3). The triangular space can be divided up into areas corresponding to different models of gene action. The markers are intended to represent a random sample of loci from the genome, and reasonable inference on the relative importance of domi-

Table 3. Estimates of allelic interactions from marker-aided studies of QTL and segregation distortion (viability loci). (Data are presented as the number of QTL or viability loci detected and whether those loci were segregating recessive or partly recessive deleterious mutations or alleles that acted in an overdominant manner.)

species	traits	QTL	(partly) recessive	overdominant	viability loci	(partly) recessive	overdominant	source
maize	grain yield	9	1	8	—	—	—	Stuber *et al.* (1992)
rice	grain yield and 11 components	37	27[a]	0	—	—	—	Xiao *et al.* (1995)
	grain yield and biomass	54[b]	5	49	—	—	—	Li *et al.* (2001)
	three grain yield components	34[b]		most	—	—	—	Luo *et al.* (2001)
Mimulus guttatus	survival to maturity	—	—	—	24[c]	3	3	Fu & Ritland (1994*a*)
Arabidopsis thaliana	viability	—	—	—	1	0	1	Mitchell-Olds (1995*a*)
Arabis petraea	survival to rosette stage, seed size, germination time, leaf number, flowering above ground and root biomass	0	0	0	6	1	4[c]	Kärkkäinen *et al.* (1999)
Pinus radiata	survival to 1 year	—	—	—	9	8	1	Kuang *et al.* (1999*b*)
Pinus taeda	embryonic lethals, survival to age 3, growth rate	2	0	2	19	16	3	Remington & O'Malley (2000*a*,*b*)

[a] The remainder of the QTL showed only additive effects.
[b] Includes both main effect and epistatic loci.
[c] The remaining viability locus or loci showed dominance or underdominance.

nance and overdominance can be drawn from a few markers of unknown linkage to the QTL, allowing for the economical application of the technique to natural populations. Five offspring from the self-fertilization of 95 *M. guttatus* grown from field-collected seed were genotyped for nine polymorphic allozyme loci. Segregation ratios differing significantly from 1 : 2 : 1 were plotted and 18 out of the 24 ratios indicated that deleterious alleles were partly or completely dominant or that loci exhibited under-dominance, a finding incompatible with either model of inbreeding depression (table 3). The investigators suggest that gametic selection rather than inbreeding depression may have resulted in the distorted segregation ratios.

A weakness of the Fu & Ritland (1994*a*) graphical technique is that it is a single-marker approach (as opposed to an interval approach; see Wang *et al.* 1999*a*). This confounds the magnitude of the effect produced by the gene with the strength of its linkage with the marker (Ritland 1996). Several maximum-likelihood and Bayesian methods for estimating segregation distortion using interval mapping have been developed (Fu & Ritland 1994*b*; Vogl & Xu 2000), but they require more marker loci than the single-marker approaches.

The adoption of *Arabidopsis thaliana* (mouseear cress) as a model system for the study of plant genetics has enabled the development of highly saturated genetic maps on a par with maize and rice (mean distance between markers *ca.* 10 cM). In a study of F_2 segregation distortion in a cross between Niederzenz and Landsberg ecotypes, Mitchell-Olds (1995*a*) used a maximum-likelihood approach to detect an overdominant viability locus on chromosome I for which homozygotes had 50% lower viability (table 3). Because this was the only locus detected, he argued that pseudo-overdominance seemed improbable because it meant that the only two loci affecting viability in this cross occupied the same 15 cM interval.

Kärkkäinen *et al.* (1999) attempted to detect both viability loci and QTL in a close relative of *A. thaliana*, *Arabis patraea* (rock cress) (table 3). Three microsatellite markers developed for *A. thaliana* were used in addition to 11 polymorphic allozyme loci to test for segregation distortion in selfed offspring (in-bud pollinations bypass the incompatibility system). The Fu & Ritland (1994*a*) graphical method and a Bayesian approach were applied to infer the mode of gene action. In examining segregation distortion to the rosette stage, six significant deviations were discovered, and both methods indicated that four of these were consistent with an overdominance model. In a test for linkage between genetic markers and QTL for six quantitative traits exhibiting significant inbreeding depression, associations were found in no greater frequency than would be expected by chance alone, suggest-

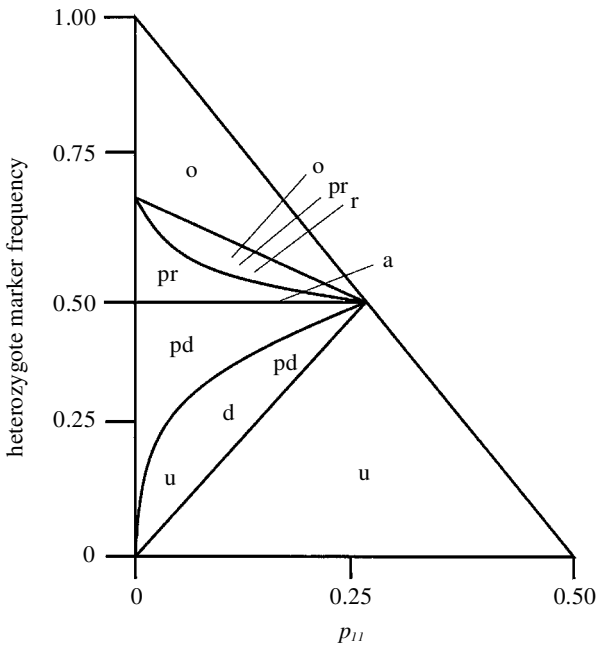

Figure 3. Graphical representation (after Fu & Ritland 1994*a*) of expected segregation ratios (the frequency of the marker heterozygote) and the less common homozygote (p_{11}) based on seven different models of allele interaction: overdominance (o), fully recessive (r), partly recessive (pr), additive (a), part dominance (pd), dominance (d) and underdominance (u). This model assumes unknown linkage between the marker and viability locus. Note the areas of overlap between the different types of allele interaction.

ing that the genome coverage by the markers was insufficient.

Large numbers of molecular markers with nearly complete genome coverage have been developed for studies of inbreeding depression in at least two pine species. In an elite breeding selection of *Pinus taeda* (loblolly pine), 226 AFLP markers were used to examine inbreeding depression acting on embryo viability (Remington & O'Malley 2000*a*) and in early life-history traits (Remington & O'Malley 2000*b*). Maximum-likelihood methods were used to establish the linkage of markers to 19 embryonic viability loci (table 3). AFLPs are dominant markers and were used because they produce unusually high numbers of variable loci. Unfortunately the use of a dominant marker left no degrees of freedom for the estimation of a dominance coefficient. Remington & O'Malley (2000*a*) tested for segregation distortion from a 3 : 1 ratio at the marker most closely linked to the QTL to qualitatively assess dominance and suggested that 16 of 19 loci affecting embryonic viability appeared to be segregating recessive deleterious alleles, with the remaining three possibly showing overdominance. Only two QTL were identified as affecting inbreeding depression for survival and early growth, but both showed signs of overdominance, though pseudo-overdominance again could not be ruled out. In a study of a selected cultivar of *P. radiata*, the segregation of 172 RAPD markers in selfed seedlings, nine viability loci were detected (Kuang *et al.* 1999*b*). Eight showed dominance or part dominance and one showed overdominance or pseudo-overdominance (table 3).

3. EPISTASIS

(a) *Biometric approaches*

Although epistasis plays an important role in many evolutionary models, including models of inbreeding depression and mating-system evolution, powerful biometric experiments for detecting epistasis within populations have been notoriously difficult to do (Fenster *et al.* 1997). Until recently the most common method for evaluating the contribution of epistasis to inbreeding depression has been to determine how fitness or fitness components change with increasing homozygosity of the genome in experimentally inbred populations. If the effect of homozygosity at a given locus is independent of the genotype at other loci (i.e. no epistasis), we expect a log-linear decline in fitness with increasing homozygosity.

Willis (1993*b*) found evidence for reinforcing epistasis in pollen viability components in a study of *M. guttatus* ranging in degree of inbreeding from $F = 0$ to $F = 0.75$, with pollen viability declining faster across the more inbred range of his experiment. Significant inbreeding depression but no epistasis was observed for the probability of flowering and the number of flowers produced. Similarly, in our analysis of two *M. guttatus* populations including plants with inbreeding coefficients varying from $F = 0$ to $F \approx 0.97$, we found no evidence of epistasis from quadratic regressions involving date of first flower, flower number, biomass, ovule production, pollen production and pollen viability (Dudash *et al.* 1997; Carr & Dudash 1997). We did, however, find evidence of reinforcing epistasis for male fertility (a function of pollen viability and pollen production) for one of these populations, and all characters showed significant variation among families in quadratic terms, indicating possible epistasis that included both reinforcing and diminishing effects within individual inbred lines. Similarly, evidence for epistasis at the population level was seen in only one of three fitness measures in *Plantago coronopus* (buck's horn plantain) (Koelewijn 1998), but epistasis within inbred lines was observed. The difficulty in interpreting these studies that involve serial inbreeding, however, is that epistatic effects and the effects of purging can become confounded (Lynch 1988).

(b) *Marker-aided approaches*

Molecular markers offer the opportunity to study the fitness of multilocus genotypes. If marker genotypes are linked to QTL, then possible epistasis can be inferred. Again, the most powerful of these QTL analyses remain in the realm of crops and particular model organisms because of the number of marker loci that need to be mapped. In their analysis of segregating generations of hybrid maize, Stuber *et al.* (1992) analysed pairwise interactions of apparent QTL by comparing the LOD from a model allowing epistasis to the LOD of a model assuming only additive gene action. Only 1% of the pairwise tests proved significant, suggesting a very minor role of epistasis. These data were re-analysed by Cockerham & Zeng (1996), however, using orthogonal contrasts, and they found significant additive × additive and dominance × dominance epistasis for 16% of QTL markers for grain yield and additive × dominant epistasis for 34%. Their test could only detect epistasis between or among linked QTL, and the pervasiveness of these effects suggested to them that multiple QTL, undetected as main effects, must exist.

The existence of multiple QTL linked to a single marker makes the repulsion linkage of deleterious alleles quite likely (pseudo-overdominance) and could easily account for their indications of overdominance for grain yield described earlier.

Digenic epistatic effects have been found to be common and widespread in the analysis of heterosis and inbreeding depression in rice (Yu *et al.* 1997; Li *et al.* 2001; Luo *et al.* 2001). These were found to have both positive and negative effects on biomass, grain yield and components of grain yield. Most of the phenotypic variance for the grain yield in F_1 crosses was explained by epistasis, and 86% of the QTL main effects were found to be involved in digenic epistatic interaction. In an earlier QTL analysis of rice, Xiao *et al.* (1995) had concluded that their data strongly favoured the dominance hypothesis of inbreeding depression for grain yield and its components. Li *et al.* (2001) suggested that it was a lack of appropriate mapping methodology that led to a failure to detect epistasis in the earlier study, and that dominance × dominance epistasis was an important component of heterosis and overdominance in rice (see Goodnight 1999).

Fu & Ritland (1996) developed a regression method to detect epistatic effects on inbreeding depression from studies of the segregation of unlinked co-dominant markers. These analyses can be more easily applied to the study of inbreeding depression in natural populations because a high density of markers and map positions are not required. Deleterious alleles are detected by segregation distortion in selfed progeny. Multilocus genotypes are then categorized by the number of markers linked to viability or fitness QTL that are homozygous. The log-fitness phenotype is then regressed against the degree of marker homozygosity, and epistasis is detected by significant deviation from linearity. The power of this technique depends on the strength of the linkage between the marker and the QTL. The approach is similar to the regression biometric technique (see § 3a) but has the advantage of minimizing the effects of purging that can confound the purely biometric approach. Using this technique with *M. guttatus*, Fu & Ritland (1996) found no evidence for epistasis in five fecundity traits but found strong evidence of reinforcing epistasis at one viability locus. Remington & O'Malley (2000*a*) found no evidence of epistasis using the same technique in their study of embryonic viability loci in *P. taeda*.

4. THE NUMBER OF LOCI AND THE DISTRIBUTION OF THEIR EFFECTS

Recessive mutations of large effect on viability and fertility have been identified in several cases. These are frequently in the form of chlorophyll-deficient mutations (Willis 1992; Kärkkäinen *et al.* 1999; Remington & O'Malley 2000*b*) or male sterility mutations (Willis 1999*c*). Studies using molecular markers have inferred recessive alleles of large effect (e.g. embryonic lethals) from segregation distortion observed in seedling cohorts (Kuang *et al.* 1999*a*,*b*). Loci with genes of large effect are not restricted to those segregating deleterious mutations. A single locus segregating alleles that behaved in an overdominant manner was found to have a large effect on viability in *Arabidopsis* (Mitchell-Olds 1995*a*) and growth rate in pine (Remington & O'Malley 2000*b*).

Despite the common observation of loci of large effect, most studies that have used molecular markers have indicated the number of loci involved in inbreeding depression or heterosis was in excess of the chromosome number of the species involved (Stuber *et al.* 1992; Xiao *et al.* 1995; Yu *et al.* 1997; Remington & O'Malley 2000*a*; Vuylsteke *et al.* 2000; Li *et al.* 2001; Luo *et al.* 2001). The ability to detect loci affecting quantitative traits is biased in favour of loci with large effects on the phenotype (Mitchell-Olds 1995*b*; Mackay 2001), and fine-scale mapping of chromosomal regions originally inferred to host only one QTL have been shown actually to contain multiple QTL (Graham *et al.* 1997; Monforte & Tanksley 2000). Clearly, all current estimates for the number of QTL are underestimates.

Overall, loci of large effect likely have only a small contribution to overall inbreeding depression. For example, Willis (1992) identified 28 loci and two different duplicate locus systems that segregated chlorophyll-deficient mutations. However, only 3.4% of individuals from two *M. guttatus* populations carried these chlorophyll-deficient mutations. Recessive mutations of large effect also appeared to affect male fertility in *M. guttatus*, and the number of carriers of recessive male sterility alleles in *M. guttatus* may be as high as 26% (Willis 1999*b*). Nevertheless, these alleles of large effect accounted for only *ca.* 31% of the inbreeding depression in male fertility and only 26% of the inbreeding depression for combined male and female fertility. The general importance of loci of small effect was revealed in an inbreeding greenhouse population of *M. guttatus* that had the opportunity to purge its load of deleterious mutations (Willis 1999*a*). Although chlorophyll-deficient and male sterility mutations were much less frequent after five generations of enforced selfing and selection among lines, male fertility and all other fitness components still showed high levels of inbreeding depression, presumably because of the ineffective removal of alleles of small effect. We observed similar patterns for biomass, flower production and fertility traits in our inbred populations of *M. guttatus* (Dudash *et al.* 1997; Carr & Dudash 1997), as did Koelewijn (1998) in *P. coronopus*.

The negative correlation between selfing rates and inbreeding depression (Husband & Schemske 1996) suggests that deleterious alleles can be purged from a population. The fact that differences in inbreeding depression between predominantly selfing and outcrossing populations is most striking at the seed and germination stages suggests that the purged mutations are largely embryonic lethals or other mutations of large effect (Husband & Schemske 1996; Byers & Waller 1999). That populations with high selfing rates can still maintain high levels of inbreeding depression (B. Charlesworth *et al.* 1990; Husband & Schemske 1996) suggests an important role of many partly recessive mutations of small effect.

5. DISCUSSION

The type of allelic interaction (dominance or overdominance), the existence of epistasis and the relative frequencies of mutations of large versus small effect are

all essential genetic elements to understanding the evolution of genetic load and mating systems (Uyenoyama *et al.* 1993). Overdominance, epistasis and deleterious alleles with individually small effects can all work together to maintain genetic load and inbreeding depression even in populations with fairly high levels of inbreeding (Charlesworth & Charlesworth 1990; Charlesworth *et al.* 1991; Barrett & Charlesworth 1991; Kondrashov 1994; Wang *et al.* 1999*b*). These are important issues not for understanding only gene flow and mating system evolution, but also for the practical conservation problems created by range fragmentation and population reductions (Fenster & Dudash 1994; Frankham 1995*a*,*b*; Hedrick & Kalinowski 2000).

There has been a growing consensus in favour of the dominance model for inbreeding depression. In their recent review of the evidence from plant and animal studies, Charlesworth & Charlesworth (1999) concluded that overdominance effects are unimportant in most cases. With a few exceptions, the data drawn from natural plant populations seem to be strongly in this camp. These data include the common (though not universal, see Byers & Waller (1999)) observation of reduced inbreeding depression in more highly selfing populations (Husband & Schemske 1996), evidence of purging in experimentally inbred populations (Barrett & Charlesworth 1991; Willis 1999*a*), variation among families in their response to serial inbreeding (Dudash *et al.* 1997; Carr & Dudash 1997; Koelewijn 1998), and biometric approaches to estimate dominance (Johnston & Schoen 1995; Dudash & Carr 1998; Willis 1999*b*).

Studies using molecular markers have frequently found evidence suggesting an important role of overdominance (though none can conclusively exclude the possibility of pseudo-overdominance). These studies (table 3) have investigated heterosis in crops (maize, Stuber *et al.* 1992; Cockerham & Zeng 1996; rice, Yu *et al.* 1997; Li *et al.* 2001; Luo *et al.* 2001) and heterosis in a cross between ecotypes of *Arabidopsis* (Mitchell-Olds 1995*a*). All of these studies involve crosses between highly divergent lines, so they may not be relevant to answering the question of which type of genetic load (mutation versus segregation) is maintained within a population. In the *Arabidopsis* case (Mitchell-Olds 1995*a*), for instance, imagining an overdominant locus in which homozygotes show a 50% reduction in viability in this highly selfing species (Abbott & Gomes 1989) seems implausible.

Molecular studies of inbreeding depression in pines (Kuang *et al.* 1999*a*,*b*; Remington & O'Malley 2000*a*,*b*) and in a natural population of *Arabis petraea* (Kärkkäinen *et al.* 1999) are probably more relevant for understanding mating system evolution. These studies provide good support for the dominance hypothesis of inbreeding depression, but all detect some loci that act in an overdominant manner. In the case of *Arabis*, only 14 markers were used to cover the genome ($2n = 16$), making the detection of multiple QTL linked in repulsion likely, so pseudo-overdominance is a probable explanation. The pine studies had much better genome coverage with well over 100 markers, but neither sets of authors rule out pseudo-overdominance. Although each of these studies has made important contributions to our understanding of the genetic basis of inbreeding depression, it is important to note that most of these dominance estimates are relevant to only a narrow class of deleterious mutations—lethals and sub-lethals. Although these deleterious mutations are common in outcrossing species, they probably account for only a small proportion of overall inbreeding depression (Willis 1999*a*).

It is clear that pseudo-overdominance has made it difficult to establish definitive evidence of true overdominance. The scepticism is somewhat understandable. In biometric studies of maize, claims of overdominance have routinely been revised to part dominance after allowing for further recombination (Crow 1999), and fine-scale mapping of putative overdominant QTL has uncovered deleterious alleles that are linked in repulsion (Graham *et al.* 1997). For understanding mating-system evolution, it may not matter whether heterozygotes gain an advantage from either true overdominance or pseudo-overdominance if the linkage between loci is tight enough. However, it is important to realize that many of the breeding designs discussed here (biometric or marker aided) generate tremendous linkage disequilibrium by crossing inbred lines. Whether such linkage disequilibrium exists in natural populations is an open question. Better understanding of gene regulation and enzyme metabolic control offers promise for unambiguous demonstration of overdominance (de Vienne *et al.* 2001).

Until recently there were few unambiguous data pointing towards an important role of epistasis in inbreeding depression, but it is clear from QTL studies of maize and rice (Cockerham & Zeng 1996; Yu *et al.* 1997; Li *et al.* 2001; Luo *et al.* 2001) that epistasis can have a huge influence. It is perhaps unsurprising that epistasis has been detected in studies of heterosis in crops whereas it has been so elusive in studies of inbreeding depression in natural populations. Epistasis is likely to have important effects in the divergence of isolated gene pools such as the various inbred lines or cultivars of crop species that are hybridized in heterosis studies (Fenster *et al.* 1997). Studies of variation in the response of individual lines to inbreeding indicate that epistasis may play a role natural populations (Pray & Goodnight 1995; Dudash *et al.* 1997; Carr & Dudash 1997; Koelewijn 1998), especially as the components of genetic variation change with increases in homozygosity (Goodnight 1999).

Until the application of molecular techniques to the study of quantitative genetic variation, the only mutations contributing to inbreeding depression that could be individually identified were those of large effect (e.g. lethals). As we have seen, these appear to account for only a small fraction of inbreeding depression. QTL analysis potentially allows the identification of loci with much smaller effects, but application of QTL analysis to natural populations has not been powerful enough to detect loci affecting quantitative traits (Kärkkäinen *et al.* 1999). As in our considerations of dominance and epistasis, the data from QTL studies of crops, which tend to show large numbers of significant QTL, may not be relevant to understanding natural populations. Even the studies on pines probably cannot be generalized because only a single genotype of each species has been examined. As far as natural populations are concerned we are left to infer that inbreeding depression is predominantly due to many loci of small effect because even though mutations of large effect

(lethals and sublethals) might be purged, deleterious mutations of small effect seem to become fixed in inbreeding lines (Dudash *et al.* 1997; Carr & Dudash 1997; Koelewijn 1998; Byers & Waller 1999; Willis 1999*a*).

In compiling the literature for this review, we were struck by the few species from natural populations for which attempts have been made to understand the genetic basis of inbreeding depression. For those species that have been examined, virtually all of the available measurements of dominance are simply average estimates; we do not yet know whether there are some loci within the genome exhibiting strong overdominance. Such loci may exist in vertebrates (see Doherty & Zingernagel 1975; Coltman *et al.* 1999; cf. Hedrick 2002) and could have extraordinary influence on mating system evolution if they exist in plants (Uyenoyama & Waller 1991). We also feel that there is still much to be learned about purging, perhaps the most important prediction of the dominance hypothesis. There have been no experimental studies of purging under field conditions, and purging under more gradual types of inbreeding (i.e. biparental inbreeding) has not been well examined. Finally, there are as yet no direct estimates of the number of loci contributing to inbreeding depression in natural populations and only weak tests of the importance of epistasis. These aspects of the genetic basis of inbreeding depression may be most tractable by the application of QTL mapping, though studies of gene regulation and metabolic control should contribute greatly to our understanding of mechanisms of overdominance and epistasis (de Vienne *et al.* 2001). Expanding on the work begun by Kärkkäinen *et al.* (1999) on outcrossing relatives of *Arabidopsis* would seem like a logical starting point.

The authors thank J. Conner, C. Ivey, T. Kennedy, C. Murren, A. Prather, L. Ries, D. Schemske and two anonymous reviewers for comments on earlier versions of this manuscript. They thank P. Crane, H. Dickinson, S. Hiscock and The Royal Society for the opportunity to do this study. This work was supported in part by National Science Foundation grant nos. DEB-0075225 to D.E.C. and DEB-9220906 to M.R.D. and USDA grant 2001-35320-10899 to M.R.D.

REFERENCES

Abbott, R. J. & Gomes, M. F. 1989 Population genetic structure and the outcrossing rate of *Arabidopsis thaliana*. *Heredity* **62**, 411–418.

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

Barrett, S. C. H. & Eckert, C. G. 1990 Variation and evolution of mating systems in seed plants. In *Biological approaches and evolutionary trends in plants* (ed. S. Kawano), pp. 229–254. London: Academic.

Britten, H. B. 1996 Meta-analyses of the association between multilocus heterozygosity and fitness. *Evolution* **50**, 2158–2164.

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.

Bustamante, C. D., Nielsen, R., Sawyer, S. A., Olsen, K. M., Purugganan, M. D. & Hartl, D. L. 2002 The cost of inbreeding in *Arabidopsis*. *Nature* **416**, 531–534.

Byers, D. L. & Waller, D. M. 1999 Do plant populations purge their genetic load? Effects of population size and mating history on inbreeding depression. *A. Rev. Ecol. Syst.* **30**, 479–513.

Carr, D. E. & Dudash, M. R. 1995 Inbreeding depression under a competitive regime in *Mimulus guttatus*: consequences for potential male and female function. *Heredity* **75**, 437–445.

Carr, D. E. & Dudash, M. R. 1996 Inbreeding depression in two species of *Mimulus* (Scrophulariaceae) with contrasting mating systems. *Am. J. Bot.* **83**, 586–593.

Carr, D. E. & Dudash, M. R. 1997 The effects of five generations of enforced selfing on potential male and female function in *Mimulus guttatus*. *Evolution* **51**, 1797–1807.

Charlesworth, B. & Charlesworth, D. 1999 The genetic basis of inbreeding depression. *Genet. Res.* **74**, 329–340.

Charlesworth, B., Charlesworth, D. & Morgan, M. T. 1990 Genetic loads and estimates of mutation rates in highly inbred plant populations. *Nature* **347**, 380–382.

Charlesworth, B., Morgan, M. T. & Charlesworth, D. 1991 Multilocus models of inbreeding depression with synergistic selection and partial self-fertilization. *Genet. Res.* **57**, 177–194.

Charlesworth, D. & Charlesworth, B. 1987 Inbreeding depression and its evolutionary consequences. *A. Rev. Ecol. Syst.* **18**, 237–268.

Charlesworth, D. & Charlesworth, B. 1990 Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. *Evolution* **44**, 870–888.

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

Cockerham, C. C. & Zeng, Z.-B. 1996 Design III with marker loci. *Genetics* **143**, 1437–1456.

Coltman, D. W., Pilkington, J. G., Smith, J. A. & Pemberton, J. M. 1999 Parasite-mediated selection against inbred soay sheep in free-living, island population. *Evolution* **53**, 1259–1267.

Comstock, R. E. & Robinson, H. F. 1952 Estimation of average dominance of genes. In *Heterosis* (ed. J. W. Gowen), pp. 494–516. Ames, IA: Iowa State Press.

Crnokrak, P. & Barrett, S. C. H. 2002 Perspective: purging the genetic load: a review of the experimental evidence. *Evolution* **56**, 2347–2358.

Crow, J. M. 1999 Dominance and overdominance. In *The genetics and exploitation of heterosis in crops* (ed. J. G. Coors & S. Pandey), pp. 49–58. Madison, WI: ASA–CSSA–SSSA Societies.

Crow, J. M. & Kimura, M. 1970 *An introduction to population genetics theory*. New York: Harper & Row.

Davenport, C. B. 1908 Degeneration, albinism and inbreeding. *Science* **28**, 454–455.

David, P. 1997 Modeling the genetic basis of heterosis: tests of alternative hypotheses. *Evolution* **51**, 1049–1057.

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

Deng, H.-W. 1998 Estimating within-locus nonadditive coefficient and discriminating dominance versus overdominance as the genetic cause of heterosis. *Genetics* **148**, 2003–2014.

de Vienne, D., Bost, B., Fiévet, J., Zivy, M. & Dillmann, C. 2001 Genetic variability of proteome expression and metabolic control. *Pl. Physiol. Biochem.* **39**, 271–283.

Doherty, P. C. & Zingernagel, R. 1975 Enhanced immunologic surveillance in mice at the H2 complex. *Nature* **256**, 50–52.

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

Dudash, M. R., Carr, D. E. & Fenster, C. B. 1997 Five generations of enforced selfing and outcrossing in *Mimulus guttatus*: inbreeding depression variation at the population and family level. *Evolution* **51**, 54–65.

East, E. M. 1908 Inbreeding in corn. *Rep. Conn. Agric. Exp. Stn* **1907**, 419–428.

Falconer, D. S. 1981 *Introduction to quantitative genetics*, 2nd edn. London: Longman.

Fenster, C. B. & Dudash, M. D. 1994 Genetic considerations for plant population restoration and conservation. In *Restoration of endangered species* (ed. M. L. Bowles & C. J. Whelan), pp. 34–62. Cambridge University Press.

Fenster, C. B., Galloway, L. F. & Chao, L. 1997 Epistasis and its consequences for the evolution of natural populations. *Trends Ecol. Evol.* **12**, 282–286.

Fisher, R. A. 1941 Average excess and average effect of a gene substitution. *Ann. Eugenics* **11**, 53–63.

Frankham, P. 1995*a* Conservation genetics. *A. Rev. Genet.* **29**, 305–327.

Frankham, P. 1995*b* Inbreeding and extinction: a threshold effect. *Conserv. Biol.* **9**, 792–799.

Fu, Y.-B. & Ritland, K. 1994*a* Evidence for the partial dominance of viability genes contributing to inbreeding depression in *Mimulus guttatus*. *Genetics* **136**, 323–331.

Fu, Y.-B. & Ritland, K. 1994*b* On estimating the linkage of marker genes to viability genes controlling inbreeding depression. *Theor. Appl. Genet.* **88**, 925–932.

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

Goodnight, C. J. 1999 Epistasis and heterosis. In *The genetics and exploitation of heterosis in crops* (ed. J. G. Coors & S. Pandey), pp. 59–68. Madison, WI: ASA–CSSA–SSSA Societies.

Graham, G. I., Wolff, D. W. & Stuber, C. W. 1997 Characterization of a yield quantitative trait locus on chromosome five of maize by fine mapping. *Crop Sci.* **37**, 1601–1610.

Hallauer, A. R. & Miranda, J. B. 1985 *Quantitative genetics in maize breeding*. Ames, IA: Iowa State University Press.

Hedrick, P. W. 2002 Pathogen resistance and genetic variation at MHC loci. *Evolution* **56**, 1902–1908.

Hedrick, P. W. & Kalinowski, S. T. 2000 Inbreeding depression in conservation biology. *A. Rev. Ecol. Syst.* **31**, 139–162.

Holsinger, K. E. 1988 Inbreeding depression doesn't matter: the genetic basis of mating-system evolution. *Evolution* **42**, 1235–1244.

Holsinger, K. E. 1993 Ecological models of plant mating systems and the evolutionary stability of mixed mating systems. In *Ecology and evolution of plant reproductive systems* (ed. R. W. Wyatt), pp. 169–191. New York: Chapman & Hall.

Holsinger, K. E. 1996 Pollination biology and the evolution of mating systems in flowering plants. *Evol. Biol.* **29**, 107–149.

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

Husband, B. C. & Schemske, D. W. 1996 Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* **50**, 54–70.

Johnston, M. O. & Schoen, D. J. 1995 Mutation rates and dominance levels of genes affecting fitness in two angiosperm species. *Science* **267**, 226–229.

Kärkkäinen, K., Kuittinen, H., Van Treuren, R., Vogl, C., Oikarinen, S. & Savolainen, O. 1999 Genetic basis of inbreeding depression in *Arabis petraea*. *Evolution* **53**, 1354–1365.

Kearsey, M. J. 1980 The efficiency of the North Carolina Expt. III and the selfing, backcrossing series for estimating additive and dominance variation. *Heredity* **45**, 73–82.

Koelewijn, H. P. 1998 Effects of different levels of inbreeding on progeny fitness in *Plantago coronopus*. *Evolution* **52**, 692–702.

Kondrashov, A. S. 1994 Muller's ratchet under epistatic selection. *Genetics* **136**, 1469–1473.

Kuang, H., Richardson, T. E., Carson, S. D., Wilcox, P. & Bongarten, B. C. 1999*a* Genetic analysis of inbreeding depression in plus tree 850.55 of *Pinus radiata* D. Don. I. Genetic map with distorted markers. *Theor. Appl. Genet.* **98**, 697–703.

Kuang, H., Richardson, T. E., Carson, S. D. & Bongarten, B. C. 1999*b* Genetic analysis of inbreeding depression in plus tree 850.55 of *Pinus radiata* D. Don. II. Genetics of viability genes. *Theor. Appl. Genet.* **99**, 140–146.

Lande, R. & Schemske, D. W. 1985 The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* **39**, 24–40.

Latta, R. & Ritland, K. 1994 The relationship between inbreeding depression and prior inbreeding among populations of four *Mimulus* taxa. *Evolution* **48**, 806–817.

Li, Z.-K. (and 10 others) 2001 Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. I. Biomass and grain yield. *Genetics* **158**, 1737–1753.

Lloyd, D. G. 1992 Self- and cross-fertilization in plants. II. The selection of self-fertilization. *Int. J. Pl. Sci.* **153**, 370–380.

Luo, L. J., Li, Z.-K., Mei, H. W., Shu, Q. Y., Tabien, R., Zhong, D. B., Ying, C. S., Stansel, J. W., Khush, G. S. & Patterson, A. H. 2001 Overdominant epistatic loci are the primary genetic basis of inbreeding depression and heterosis in rice. II. Grain yield components. *Genetics* **158**, 1755–1771.

Lynch, M. 1988 Design and analysis of experiments on random drift and inbreeding depression. *Genetics* **120**, 791–807.

Lynch, M. & Walsh, B. 1998 *Genetics and analysis of quantitative traits*. Sunderland, MA: Sinauer.

McCall, C., Waller, D. M. & Mitchell-Olds, T. 1994 Effects of serial inbreeding on fitness components in *Impatiens capensis*. *Evolution* **48**, 818–827.

Mackay, T. F. C. 2001 The genetic architecture of quantitative traits. *A. Rev. Genet.* **35**, 303–339.

Mitchell-Olds, T. 1995*a* Interval mapping of viability loci causing heterosis in *Arabidopsis*. *Genetics* **140**, 1105–1109.

Mitchell-Olds, T. 1995*b* The molecular basis of quantitative genetic variation in natural populations. *Trends Ecol. Evol.* **10**, 324–328.

Monforte, A. J. & Tanksley, S. D. 2000 Fine mapping of a quantitative trait locus (QTL) from *Lycopersicon hirsutum* chromosome 1 affecting fruit characteristics and agronomic traits: breaking linkage among QTLs affecting different traits and dissection of heterosis for yield. *Theor. Appl. Genet.* **100**, 471–479.

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.

Pray, L. A. & Goodnight, C. J. 1995 Genetic variation in inbreeding depression in the red flour beetle *Tribolium castaneum*. *Evolution* **49**, 176–188.

Remington, D. L. & O'Malley, D. M. 2000*a* Whole-genome characterization of embryonic stage inbreeding depression in a selfed loblolly pine family. *Genetics* **155**, 337–348.

Remington, D. L. & O'Malley, D. M. 2000*b* Evaluation of major genetic loci contributing to inbreeding depression for survival and early growth in a selfed family of *Pinus taeda*. *Evolution* **54**, 1580–1589.

Ritland, K. 1996 Inferring the genetic basis of inbreeding depression in plants. *Genome* **39**, 1–8.

Schemske, D. W. & Lande, R. 1985 The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* **39**, 41–52.
Schoen, D. J., Morgan, M. T. & Bataillon, T. 1996 How does self-pollination evolve? Inferences from floral ecology and molecular genetic variation. *Phil. Trans. R. Soc. Lond.* B **351**, 1281–1290.
Shull, G. H. 1908 The composition of a field of maize. *Rep. Am. Breed. Assoc.* **4**, 296–301.
Simmonds, N. W. 1981 *Principles of crop improvement*. London: Longman.
Slate, J. & Pemberton, J. M. 2002 Comparing molecular measures for detecting inbreeding depression. *J. Evol. Biol.* **15**, 20–31.
Smouse, P. E. 1986 The fitness consequences of multiple-locus heterozygosity under the multiplicative overdominance and inbreeding depression models. *Evolution* **40**, 946–958.
Stebbins, G. L. 1974 *Flowering plants: evolution above the species level*. Cambridge, MA: Harvard University Press.
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.
Uyenoyama, M. K. & Waller, D. M. 1991 Coevolution of self-fertilization and inbreeding depression. II. Symmetric overdominance in viability. *Theor. Popul. Biol.* **40**, 47–77.
Uyenoyama, M. K., Holsinger, K. E. & Waller, D. M. 1993 Ecological and genetic factors directing the evolution of self-fertilization. *Oxf. Surv. Evol. Biol.* **9**, 327–381.
Vogl, C. & Xu, S. 2000 Multipoint mapping of viability and segregation distorting loci using molecular markers. *Genetics* **155**, 1439–1447.
Vogler, D. W. & Kalisz, S. 2001 Sex among the flowers: the distribution of plant mating systems. *Evolution* **55**, 202–204.
Vuylsteke, M., Kuiper, M. & Stam, P. 2000 Chromosomal regions involved in hybrid performance and heterosis: their AFLP®-based identification and practical use in prediction models. *Heredity* **85**, 208–218.
Wang, D. L., Zhu, J., Li, K. & Patterson, A. H. 1999*a* Mapping QTL with epistatic effects and QTL × environment interactions by mixed model approaches. *Theor. Appl. Genet.* **99**, 1255–1264.
Wang, J. L., Hill, W. G., Charlesworth, D. & Charlesworth, B. 1999*b* Dynamics of inbreeding depression due to deleterious mutations in small populations: mutation parameters and inbreeding rate. *Genet. Res.* **74**, 165–178.
Weir, B. S. & Cockerham, C. C. 1973 Mixed self and random mating at two loci. *Genet. Res.* **21**, 247–262.
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*a* Partial self-fertilization and inbreeding depression in two populations of *Mimulus guttatus*. *Heredity* **71**, 145–154.
Willis, J. H. 1993*b* Effects of different levels of inbreeding on fitness components in *Mimulus guttatus*. *Evolution* **47**, 864–876.
Willis, J. H. 1999*a* The role of genes of large effect on inbreeding depression in *Mimulus guttatus*. *Evolution* **53**, 1678–1691.
Willis, J. H. 1999*b* Inbreeding load, average dominance and the mutation rate for mildly deleterious alleles in *Mimulus guttatus*. *Genetics* **153**, 1885–1898.
Willis, J. H. 1999*c* The contribution of male-sterility mutations to inbreeding depression in *Mimulus guttatus*. *Heredity* **83**, 337–346.
Wright, S. 1921 Systems of mating. II. The effects of inbreeding on the genetic composition of a population. *Genetics* **6**, 124–143.
Xiao, J., Li, J., Yaun, L. & Tanksley, S. D. 1995 Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. *Genetics* **140**, 745–754.
Yu, S. B., Li, J. X., Xu, C. G., Tan, Y. F., Gao, Y. J., Li, X. H., Zhang, Q. & Maroof, M. A. S. 1997 Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. *Proc. Natl Acad. Sci. USA* **94**, 9226–9231.
Ziehe, M. & Roberds, J. H. 1989 Inbreeding depression due to overdominance in partially self-fertilizing plant populations. *Genetics* **121**, 861–868.

Discussion

M. W. Bayliss (*Biotechnology Consultant, Wokingham, UK*). The heterosis produced from crossing inbred lines has considerable agricultural importance and probably has a similar genetic basis to inbreeding depression. Maize breeders have spent 80 years trying to understand heterosis, including intensive use of molecular tools since these became available in the 1980s. However, there is still no generally accepted method that can predict specific combining ability, so it might require a considerable amount of work to understand the causes of inbreeding depression.

D. E. Carr. I agree.

GLOSSARY

AFLP: amplified fragment length polymorphism
LOD: log-likelihood ratio score
QTL: quantitative trait loci
RAPD: random amplified polymorphic DNA
RFLP: restriction fragment length polymorphism
RIL: recombinant inbred line