Interval Mapping of Viability Loci Causing Heterosis in Arabidopsis

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ABSTRACT

The genetic basis of heterosis has implications for many problems in genetics and evolution. Heterosis and inbreeding depression affect human genetic diseases, maintenance of genetic variation, evolution of breeding systems, agricultural productivity, and conservation biology. Despite decades of theoretical and empirical studies, the genetic basis of heterosis has remained unclear. I mapped viability loci contributing to heterosis in Arabidopsis. An overdominant factor with large effects on viability mapped to a short interval on chromosome *I*. Homozygotes had 50% lower viability than heterozygotes in this chromosomal region. Statistical analysis of viability data in this cross indicates that observed viability heterosis is better explained by functional overdominance than by pseudo-overdominance. Overdominance sometimes may be an important cause of hybrid vigor, especially in habitually inbreeding species. Finally, I developed a maximum likelihood interval mapping procedure that can be used to examine chromosomal regions showing segregation distortion or viability selection.

HETEROSIS is central to many questions in evolutionary and applied genetics. Increased fitness or yield of heterozygotes (the phenomenon of hybrid vigor or heterosis) contributes to high yield in many crop species and may maintain genetic variation in natural populations. Despite their importance, the factors causing hybrid vigor and hybrid breakdown are unknown at the genetic and molecular level.

Evolutionary biologists have proposed several models to explain inbreeding depression and heterosis, invoking dominance (masking of deleterious recessives), overdominance (single locus heterosis), or epistasis (traits derived from two different lines give superior performance in combination) (MITCHELL-OLDS and WALLER 1985). Few examples of true overdominance have been documented in any organism (CAVALLI-SFORZA and BODMER 1971; POGSON 1991).

The basis of genetic load has been inferred from biometric techniques. Experiments examining the genetic basis of heterosis or inbreeding depression may evaluate the correspondence of population data with a particular model of heterosis (*e.g.*, BARRETT and CHARLESWORTH 1991). However, estimates of the average levels of dominance over all loci may provide little information on the possible role of overdominance at individual loci. In contrast, linkage studies are not limited to an estimate of overall dominance or overdominance. Even with molecular markers, it is difficult to obtain clear evidence for overdominant gene action (STUBER *et al.* 1992), because heterotic chromosome segments could result from true overdominance or pseudo-overdominance caused by tightly linked dominant loci in repulsion phase, also known as associative overdominance. Fine-scale mapping is essential for understanding the genetic basis of heterosis.

If inbreeding depression results from major deleterious alleles, then gradual inbreeding and selection can eliminate these alleles, leaving little permanent inbreeding depression (*e.g.*, TEMPLETON 1986). Alternatively, if slightly deleterious alleles are responsible for inbreeding depression, then it may be impossible to eliminate inbreeding depression (BARRETT and CHARLESWORTH 1991). Studies of heterosis in Drosophila have used balanced lethal chromosomes to compare individuals either homozygous or heterozygous for particular chromosomes. These reveal that the viability load is attributable to lethal chromosomes or detrimental effects in chromosomal homozygotes (SIMMONS and CROW 1977; CROW and SIMMONS 1983). However, because quantitative genetic methods cannot distinguish between deaths attributable to few loci of large effect or those due to deleterious alleles at many loci with a net lethal effect (synthetic lethals) (LEWONTIN 1974), it is difficult to interpret the results of biometric studies. Synthetic lethals resulting from epistatic interactions among regulatory loci have been documented in Arabidopsis (DENG and ANG 1994). Although major deleterious recessive alleles (*e.g.*, lethals or semilethals) may be responsible for most of the genetic load in Drosophila (LEWONTIN 1974; SIMMONS and CROW 1977; CROW and SIMMONS 1983), they do not explain heterosis in highly inbred species such as Arabidopsis (GRIFFING and LANGRIDGE 1963; PEDERSON 1968; GRIFFING and ZSIROS 1971), where major deleterious alleles will be eliminated after inbreeding.

This article develops statistical methods for interval mapping of loci causing segregation distortion or viability differences. This procedure is applied to molecular

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TABLE 1

Expected probabilities for each two-locus marker genotype with heterozygote viability $1 + h$

Genotype	Probability
AABB	$\frac{1}{4}(1-r)^2(1-s)^2 + \frac{1}{2}(1+h)rs(1-r)(1-s) + \frac{1}{4}r^2s^2$
AABb	$\frac{1}{2}(1-r)^2 s(1-s) + (1+h)(\frac{1}{2}r(1-s)^2(1-r) + \frac{1}{2}rs^2(1-r)) + \frac{1}{2}r^2(1-s)s$
AAbb	$\frac{1}{4}(1-r)^2s^2 + \frac{1}{2}(1+h)rs(1-r)(1-s) + \frac{1}{4}r^2(1-s)^2$
AaBB	$\frac{1}{2}r(1-s)^2(1-r) + (1+h)(\frac{1}{2}(1-r)^2 s(1-s) + \frac{1}{2}r^2(1-s)s) + \frac{1}{2}rs^2(1-r)$
AaBb	$2rs(1-r)(1-s) + (1+h)(\frac{1}{2}(1-r)^2(1-s)^2 + \frac{1}{2}(1-r)^2 s^2 + \frac{1}{2}r^2s^2 + \frac{1}{2}r^2(1-s)^2)$
Aabb	$\frac{1}{2}r(1-s)^2(1-r) + (1+h)(\frac{1}{2}(1-r)^2 s(1-s) + \frac{1}{2}r^2(1-s)s) + \frac{1}{2}rs^2(1-r)$
aaBB	$\frac{1}{4}(1-r)^2 s^2 + \frac{1}{2}(1+h)rs(1-r)(1-s) + \frac{1}{4}r^2(1-s)^2$
aaBb	$\frac{1}{2}(1-r)^2 s(1-s) + (1+h)(\frac{1}{2}r(1-s)^2(1-r) + \frac{1}{2}rs^2(1-r)) + \frac{1}{2}r^2(1-s)s$
aabb	$\frac{1}{4}(1-r)^2(1-s)^2 + \frac{1}{2}(1+h)rs(1-r)(1-s) + \frac{1}{4}r^2s^2$

To compute the frequency of each marker genotype, divide each element by the population mean fitness, $1 + h/2$.

marker data to map heterotic chromosome segments with large heterotic effects on viability.

MATERIALS AND METHODS

Marker data: Data from 190 molecular markers in 451 individuals in three F2 crosses (Niederzenz × Landsberg, Niederzenz × Columbia, and Columbia × Landsberg) were obtained from AATDB, the Arabidopsis database (CHERRY *et al.* 1992). Some individuals were not genotyped for some markers, so the mean distance between scored markers was ~10 cM.

Interval mapping of loci causing segregation distortion: Consider a locus, Q, that deviates from Mendelian segregation, located somewhere within an interval between two molecular markers, A and B. Let r and s be the recombination fractions between A and Q and between Q and B, respectively. At the Q locus, the relative viabilities of homozygotes and heterozygotes are 1 and $1 + h$. Molecular markers A and B are scored on F2 progeny from an AAQQBB × aaqqbb cross. Let $\mathbf{N}$ and $\mathbf{G}$ be 9×1 vectors. Elements N_i contain the number of observed individuals with each two-locus genotype at the A and B loci. Elements G_i are the probabilities of each marker genotype given Mendelian segregation, recombination fractions r and s, and viability difference h (Table 1). At several points in the interval between A and B (*e.g.*, each 1 cM), we test for a putative viability locus that may cause deviations from Mendelian segregation. At each putative viability gene, r and s are known from the linkage map and h can be estimated by maximum likelihood. The likelihood of the observed data is

$$Pr(\mathbf{N} \mid h) = \frac{(\Sigma N_i)!}{\Pi N_i!} \prod G_i^{N_i},$$

with G_i taken from Table 1. We seek the maximum likelihood estimator, $\hat{h}$, that results in maximum $Pr(N \mid h)$. A statistical test for the presence of a heterotic viability gene is given by $2 \log_e (L_1/L_0)$, where L_1 is the maximized likelihood of the data allowing for viability differences and L_0 is the likelihood under the null hypothesis that $h = 0$. This likelihood ratio test is asymptotically distributed as a 1 d.f. χ^2 (WILKS 1938). Equivalently, the LOD score (from the base 10 *l*og of the *od*ds) equals $\log_{10} (L_1/L_0)$. $2 \times \log_e (10) \times$ LOD is asymptotically distributed as χ^2.

For the current data, tests of statistical significance used a LOD threshold of 2.69. LANDER and BOTSTEIN (1989) recommend a 1 d.f. significance threshold of LOD 2.25 in a single cross for 10-cM marker spacing and five 100-cM chromosomes (see below). After a Bonferoni correction for three crosses, this gives an experimentwide significance threshold of 2.69. The conservative Bonferoni procedure protects against spurious inference of loci influencing viability in these crosses (RICE 1989). To verify the type I error rate produced by this significance threshold, we simulated experiments similar to our data, with 150 individuals in each of three populations, 10 cM between each measured marker, and no actual segregation distortion. We recorded the percentage of simulations where the number of heterozygotes *vs.* homozygotes at the most deviant marker out of all three populations resulted in LOD > 2.69.

When directional selection was absent in the Arabidopsis data, heterosis was tested against a null hypothesis of equal numbers of heterozygotes and homozygotes. The segregation distortion interval mapping procedure was implemented in Pascal, with a grid-search maximization algorithm within intervals. When considering a putative QTL located at a molecular marker, QTL genotype was known with certainty, and LOD was computed by comparing observed and expected numbers of heterozygotes at each marker.

RESULTS

Simulations showed that the LANDER–BOTSTEIN–BONFERONI significance threshold was remarkably accurate. Out of 3000 simulated experiments, each with 150 plants in each of three populations, the putative 5% significance threshold (2.69) was exceeded 5.4% of the time. Therefore, 2.69 is taken as an approximate 5% significance threshold, supported by both analytical argument and Monte Carlo simulation.

An overdominant factor with large effects on viability mapped on chromosome *1* in Arabidopsis. Figure 1 shows a symmetrically overdominant chromosome region near 84 cM on chromosome *I*. In F2 progeny from Niederzenz × Landsberg, homozygotes had 50% lower viability than heterozygotes at marker 1511a, as shown by a significant deficit of homozygotes (LOD = 2.73, $N = 111$), but there is no directional segregation distor-

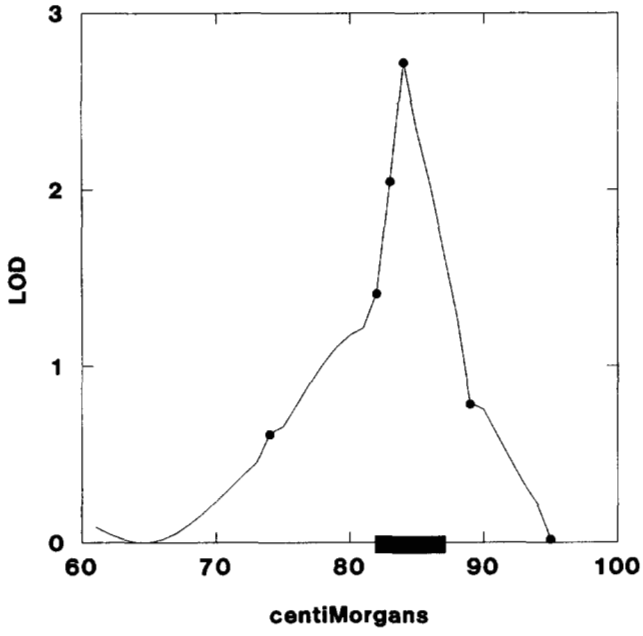

FIGURE 1.—An overdominant factor with large effects on viability maps near 84 cM on chromosome *I* in Niederzenz × Landsberg. Homozygotes have 50% lower viability than heterozygotes at marker 1511a. High LOD shows statistical deviation of nearby markers from a Mendelian model of inheritance. ●, position of molecular markers; ■, 1 LOD confidence interval.

tion (allele frequency = 0.51). The 1 and 2 LOD confidence intervals containing this heterotic locus are 5 and 15 cM wide, respectively.

I tested whether inversion-induced crossover suppression might cause pseudo-overdominance on chromosome *I*. Mapmaker (LANDER *et al.* 1987) showed that markers in the Niederzenz × Landsberg cross were unambiguously ordered in agreement with the Arabidopsis consensus map. Consequently, there is no evidence for suppression of recombination in this region.

A total of eight loci experiencing directional selection were detected in the Columbia × Niederzenz and Columbia × Landsberg crosses (not shown). These deleterious alleles may be artifacts of past mutagenesis. Therefore, these data cannot address the importance of dominant or partially dominant viability factors in natural populations.

DISCUSSION

Genetic basis of heterosis: An overdominant factor with large effects on viability mapped to a short interval on chromosome *I*. Homozygotes had 50% lower viability than heterozygotes in this chromosomal region. Several nonexclusive models may explain inbreeding depression and heterosis, attributable to dominance (masking of deleterious recessives), overdominance (single locus heterosis), or epistasis (UYENOYAMA *et al.* 1993). Often, heterozygous individuals display greater fitness than more homozygous individuals (*e.g.*, KOEHN *et al.* 1988; ZOUROS *et al.* 1988). This pattern may be due to either functional overdominance, where heterozygosity of a particular gene causes increased fitness, or pseudo-overdominance, caused by deleterious recessive alleles in repulsion phase (MITTON and GRANT 1984). Most quantitative genetic studies of heterosis provide little support for overdominant effects on fitness (CHARLESWORTH and CHARLESWORTH 1987; HOULE 1989; BARRETT and CHARLESWORTH 1991). However, quantitative genetic studies cannot easily distinguish among models of heterosis, because effects of individual loci cannot be estimated. Also, overdominance may be difficult to detect when heterosis is attributable to several genetic mechanisms. For these reasons, quantitative genetic studies are not empirically sufficient to rule out an overdominant contribution to heterosis.

Although short overdominant chromosome segments might result from pseudo-overdominance of tightly linked loci, the present data provide little support for this mechanism. In the Niederzenz × Landsberg cross, no marker loci experienced significant directional selection. Thus, the pseudo-overdominant hypothesis must postulate that there are only two deleterious recessive genes in a 500-cM genome (CHANG *et al.* 1988), both located within a small chromosomal region (say 15 cM, the 2 LOD confidence interval). If such genes are located randomly on the linkage map, as would occur if recombination occurs near expressed genes (DOONER *et al.* 1991), then the pseudo-overdominance hypothesis requires an improbable clustering of deleterious recessive loci in repulsion phase: *Pr*(pseudo-overdominance)

$$\frac{\text{two loci in 15 cM}}{\text{500-cM genome}} \times Pr(\text{loci in repulsion})$$

$$= \frac{15}{500} \times \frac{1}{2} = 0.015.$$

This estimated probability should be taken as a rough approximation due to imprecision in fine-scale mapping. Clearly, however, this observed viability heterosis is better explained by functional overdominance.

Although overdominant loci are unlikely to segregate in highly inbred natural populations of Arabidopsis, overdominance may contribute to heterosis in this hybrid population. Overdominant chromosome segments affecting yield have recently been found in maize (STUBER *et al.* 1992), an outcrossing species. In that study, nine regions affecting yield were mapped, and eight of these showed significant overdominance. This might arise from genes in repulsion phase that affect different components of yield or from true overdominance. Even if deleterious recessive alleles are the main cause of genetic load, existence of a single overdominant locus can dominate the dynamics of breeding system evolution (UYENOYAMA *et al.* 1993). Overdominance may be relatively more important in partially

inbred species, because major deleterious recessives make little contribution to genetic load and minor deleterious genes can be purged by gradual inbreeding (LANDE 1988). These results emphasize that inbreeding depression may remain in highly inbred species (CHARLESWORTH *et al.* 1990) or small populations of rare organisms (HAMILTON and MITCHELL-OLDS 1994) and may have important implications for species management in conservation biology.

Finally, quantitative genetic studies (GRIFFING and LANGRIDGE 1963; PEDERSON 1968; GRIFFING and ZSIROS 1971) suggest that Arabidopsis may display marginal overdominance, such that heterozygotes are favored when fitness is averaged across several environments. Additional experiments are needed in this area.

Crosses between ecotypes of *Arabidopsis thaliana* permitted mapping of loci with large effects on viability, displaying 50% mortality of disfavored genotypes. This supports recent suggestions that evolution of local adaptation may sometimes involve genes with large effects (ORR and COYNE 1992; MITCHELL-OLDS 1995). Arabidopsis provides a tractable system to elucidate the molecular genetic basis of heterosis.

Statistics of interval mapping: Maximum likelihood interval mapping can be used to identify chromosomal regions showing segregation distortion or viability selection. There has been considerable recent discussion regarding appropriate statistical thresholds when many markers result in a large number of partially correlated statistical tests (FEINGOLD *et al.* 1993; SOUKRI and LATHROP 1993; CHURCHILL and DOERGE 1994; MANGIN and GOFFINET 1994) or when QTL location parameters (*e.g.*, r and s) are irrelevant under the null hypothesis (DAVIES 1977). Permutation procedures that reshuffle phenotypes with respect to marker genotypes (CHURCHILL and DOERGE 1994) cannot be applied to this data set, because only living plants were genotyped in this study. However, the LANDER–BOTSTEIN–BONFERONI significance threshold gave excellent results in Monte-Carlo simulations.

It was also reassuring that the LOD map (Figure 1) gave congruent results within intervals and at adjacent markers. This result contrasts with the "LOD humps" typically observed in interval mapping of QTL affecting metrical traits (*e.g.*, PATERSON *et al.* 1991; STUBER *et al.* 1992). Many such studies show that maximum LOD occurs within intervals, where our ability to predict QTL genotypes is poorest, and LOD scores decline sharply at markers, where codominant markers give complete information on QTL genotypes. These LOD humps may represent statistical artifacts caused by assumption violations. In particular, QTL elsewhere in the genome may cause residuals to be a mixture of normals, so that maximum likelihood mixture models cause LOD humps in the interval between markers (T. MITCHELL-OLDS, unpublished data). A related explanation was suggested by PATERSON *et al.* (1991, p. 184), who noted that an interval that does not contain an actual QTL may show a LOD maximum near the center of the interval, "since this position is farthest from any potentially conflicting data at the observed markers." However, LOD humps within intervals are also observed in single d.f. least-squares interval mapping (MITCHELL-OLDS 1995). These least-squares models apply the same statistical model at all points along a chromosome, and they cannot recognize mixtures of normals caused by unlinked QTL. The factors that cause LOD humps in least squares interval mapping are not clear.

For these reasons, it is worth emphasizing that genetic information about QTL is most complete at a molecular marker. Within an interval our ability to predict QTL genotypes is always reduced. In some instances LOD humps within intervals may represent statistical artifacts. A putative QTL within an interval can be verified by scoring new molecular markers within the region of interest. Also, subsequent independent experiments can test a given genomic region with lower statistical thresholds that are not protected for multiple statistical tests, thereby gaining greater statistical power.

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