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Synergistic epistasis between loci affecting fitness: evidence in plants and fungi

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Summary

Information on the nature of epistasis between alleles affecting fitness is hardly available, but relevant for, among other issues, our understanding of the evolution of sex and recombination. Evidence of synergistic epistasis between deleterious mutations is support for the Mutational Deterministic hypothesis of the evolution of sex, while finding antagonistic epistasis between beneficial alleles would support the Environmental Deterministic hypotheses. Both types of epistasis are expected to cause negatively skewed fitness distributions of full-sib offspring from a sexual cross. Here, we have studied the form of the distribution of a variety of quantitative characters related to fitness by searching the literature. The fitness traits encountered include the mycelial growth rate in fungi, and earliness, resistance against pathogens, seed number, and pollen fitness in plants. Fitness-related traits in plants show almost exclusively negative skewness, while the results for fungal species are more ambiguous. Possible sources of negative skewness other than epistasis, such as recessiveness of deleterious alleles or a negatively skewed error variance, were tested and found to be unimportant. We argue that these results suggest the existence of synergistic epistasis between deleterious alleles or antagonistic epistasis between beneficial alleles in plants, which is general support for the currently popular hypotheses of sex and recombination, but does not distinguish between them.

1. Introduction

The nature of epistasis between alleles that affect fitness is largely unknown, but very relevant for, among other things, the continuing debate on the evolution of sex (e.g. Hurst & Peck, 1996). The currently popular hypotheses of sex and recombination rely on either synergistic epistasis between deleterious mutations (the Mutational Deterministic hypothesis: Crow, 1970; Kondrashov 1982, 1988; Charlesworth, 1990) or antagonistic epistasis between beneficial mutations (the Environmental Deterministic hypotheses: Maynard Smith, 1980, 1988; Hamilton *et al.*, 1990; Charlesworth, 1993; Barton, 1995; Kondrashov & Yampolsky, 1996). Since both types of epistasis can be described by the same concave function relating numbers of alleles and log fitness, both classes of models can be unified and modelled in the same way (Barton, 1995; Charlesworth & Barton, 1996).

Recently, we proposed a new experimental test for the nature of epistasis between alleles affecting fitness (De Visser *et al.*, 1997*a*). This test considers the skewness of the log fitness distribution among the offspring of a sexual cross between two parents. Since every allele affecting fitness in the parents is inherited by an equal fraction of the offspring, skewness of the log fitness distribution reveals the prevailing type of epistasis: negative skewness indicates synergistic epistasis between deleterious alleles (or antagonistic epistasis between beneficial alleles), while positive skewness indicates antagonistic epistasis between deleterious alleles (or synergistic epistasis between beneficial alleles). Finding negative skewness would, therefore, be support for both classes of hypotheses on the evolution of sex.

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The notion that skewness of a phenotypic distribution may provide information on interaction between genes affecting this phenotype appears to be old. In the 1930s, several authors interested in quantitative characters of plants such as fruit size or age at maturity, found skewed distributions in crosses between different races, which they related to non-additive gene action (Rasmusson, 1933; Powers, 1936; Sinnot, 1937; MacArthur & Butler, 1938). For example, genes affecting fruit weight in *Cucurbita pepo* (Sinnot, 1937) and *Lycopersicon* (MacArthur & Butler, 1938) appeared to have multiplicative effects, which was concluded from the positively skewed distribution of F_1 and F_2 fruit weights and the roughly normal distribution of the log of fruit weight.

In this paper, we use the skewness test (De Visser *et al.*, 1997*a*) to search the literature for empirical evidence of the nature of epistasis between loci affecting fitness in various organisms. However, the skewness test was originally developed for haploid organisms. In such organisms, the genetic contribution to the fitness distribution is exclusively determined by allelic effects and by interaction between loci (i.e. non-allelic interaction). In diploid organisms, dominance (i.e. allelic interaction) may affect the form of the distribution as well. Dominance may not be very important for the form of the phenotypic distribution if many loci with small effect are involved (Rasmusson, 1933), but its effect relative to that of non-allelic interaction needs further study. An interesting opportunity for applying the skewness test to diploids is provided by studies that use recombinant-inbred lines or doubled-haploid lines for the construction of genomic maps. Recombinant inbred lines are F_1 recombinants that have been inbred during a number of generations (via selfing or sib-mating) to render most loci homozygous, while doubled-haploids have been made homozygous at all loci (e.g. Simpson & Snape, 1981). The relative contribution of dominance to the skewness observed can be studied by comparing progenies with different levels of homozygosity.

Critical to the skewness test is knowledge of the form of the relationship between the fitness-related trait studied and total fitness. Synergistic epistasis between deleterious alleles found at the level of the fitness trait may result in antagonistic epistasis at the level of total fitness if, for instance, total fitness would relate to the fitness trait by an exponentially increasing relationship. Unfortunately, for most fitness-related traits their relation to total fitness is still poorly understood. Furthermore, differences in experimental protocols and scale of measurement obscure the quantitative interpretation of the fitness-related trait involved. Our approach, therefore, was to include all relevant fitness-related traits encountered in our search, by leaving the original scale of measurement intact and assuming a linear relationship between each trait and fitness. The effect of deviations from a linear relationship between fitness-related trait and total fitness on our conclusions is discussed.

2. Methods

The phenotypic distributions used in this study were gathered by searching the literature, with emphasis on the plant breeding literature. The fungal data came from Caten (1979) and references therein. For the plant data, we searched recent issues (1995 and 1996) of *Theoretical and Applied Genetics* and references therein. The following criteria were used: (1) only full-sib progenies, i.e. offspring from two parents, were used; (2) the quantitative character involved should be a fitness-related trait, i.e. should affect survival and/or fecundity; (3) the character should be quantitatively inherited; studies mentioning the segregation of genes at only one or two loci and studies showing clearly bimodal distributions were excluded; (4) distributions should represent random samples of the offspring.

In order to obtain an estimate of the skewness of a distribution, the length of the columns in the frequency distributions was measured in half millimetres (on photocopies of at least 50% the original size). Next, the logarithm of the mean value of each phenotypic class was calculated, since skewness of the log fitness distribution reflects the relevant type of epistasis (Kondrashov, 1988; Charlesworth, 1990). However, in some data sets the value zero or negative values were included, which made log-transformation impossible. A transformation such as $\log(x+c)$, where c is a constant, is inappropriate because it would affect the skewness. Therefore, the skewness of the untransformed data was also measured. Negative skewness at the original scale would be even more negative at a log scale, and thus provides conservative evidence for synergism. The significance of the skewness statistic (g_1) was tested with a two-tailed t-test, using the exact formula for the standard error of g_1 in cases with sample size smaller than 100; otherwise the approximation was used (Sokal & Rohlf, 1981, p. 139). To correct for the fact that multiple tests were performed, we used the conservative sequential Bonferroni method (Rice, 1988).

We assumed a linear relationship between a character and fitness. Two exceptions were made for different reasons. First, the character *mycelial growth rate* was originally given as a one-dimensional rate of increase (radial or linear growth rate). However, a better estimate of the fitness of a fungus seems to be the rate of spore production, and spore number has been found to show a highly significant positive correlation with mycelial surface area (De Visser *et al.*, 1997*b*). Since the logarithm of the rate of

increase of mycelial surface area (i.e. log fitness) is proportional to the untransformed rate of increase of the one-dimensional colony diameter, we used this character on the original linear scale as a representation of fitness at a log scale. Fitness at a linear scale was obtained for this character by raising the original data to the power 2. Taking the logarithm of the original one-dimensional growth data would result in more negatively skewed distributions. Second, the characters *earliness* and *resistance* were originally given in *days to flowering* or *days to maturity* and some index of host infection, respectively, which are inversely related to fitness. Therefore, a translation into the fitness components *earliness* and *resistance* was needed. In order to leave the original scale intact, *days to flowering* and *level of infection* were mirrored in the mean value of each data set, i.e. the following transformation was performed: $\log(x_{\text{mean}} - x_i)$, where x_i and x_{mean} are the individual and the mean of all values at the original scale of measurement, respectively.

Where possible, an indication of the relative genetic contribution to the total phenotypic variance was given. The higher the genetic contribution, the smaller the effect a possibly skewed error variance can have on the distribution of mean values. As an estimate of the relative genetic contribution, the value of the broad-sense heritability, $h^2_{\text{B}} = V_{\text{G}}/V_{\text{tot}}$, was given where possible. However, in some studies only an estimate of the fraction of the total variance that could be explained with a number of significant quantitative trait loci (QTLs) was presented. This estimate provided a lower limit for the broad-sense heritability (indicated by '$>$' in Table 1).

3. Results

Table 1 presents the skewness of fitness-related traits encountered in the literature. All data on haploids refer to fungal species, while all data on diploids are exclusively from plants. Studies involving animals either did not concern fitness traits, or did not present distributions (e.g. Shook *et al*., 1996). In 15 crosses, skewness was significantly negative at the appropriate log scale after Bonferroni correction for multiple testing (14 crosses showed negative skewness at the untransformed scale), while only three crosses showed positive skewness (four crosses did so as the original scale). Seventeen crosses did not show significant skewness at the log scale (versus 26 crosses at the original scale).

(i) *Fungi*

The only fitness-related trait for which accurate data were found in haploids, was the mycelial growth rate for a number of fungal species. Both signs of skewness were found for this character at log scale, although after correcting for multiple testing, only the positive skewness of one *Aspergillus* cross was significant. At the untransformed scale, two *Aspergillus* crosses showed significant positive skewness. Mycelial growth rate in *Aspergillus* was measured on circular colonies on plates (Jinks *et al*., 1966; Caten, 1979), while in the other three species it was measured as linear growth in special growth tubes (Croft & Simchen, 1965; Simchen, 1966; Paper *et al*., 1967). Therefore, it is unclear whether the difference in skewness for *Aspergillus* and the other three fungal species is due to a difference in methodology of measurement or to a difference in epistasis between different species or crosses.

(ii) *Plants*

All diploid data concern plant species, for which a relatively large number of examples was found in the plant breeding literature on the characters earliness and resistance against pathogens. For earliness, all crosses that revealed significantly skewed distributions showed negative skewness. The only study showing no skewness for earliness involved *Brassica oleracea*, which had a flowering time index that included the proportion of plants of a line that flowered at a given time (Camargo & Osborn, 1996) instead of 'days to flowering' as used in all other studies. Also, at the untransformed scale the only significant skewness observed was negative (in three crosses), which emphasizes the robustness of this result.

For resistance against pathogens, of the seven crosses showing significant skewness, only two were positive at the log scale (2 of 10 at the linear scale). For resistance against *Pyricularia oryzae* in *Oryza* and against *Verticillium* in *Solanum*, only a skewness measure of the untransformed data is available. The significantly negative skewness at this scale suggests that skewness will certainly be negative at the log scale. The two crosses showing significantly positive skewness were different from the other studies on resistance in at least three respects. First, the resistances concerned in all other studies were against micro-organisms (mostly fungi, a bacterium and a nematode), while the two crosses showing antagonism concerned resistance against an insect, the bean weevil (*Acanthoscelides obtectus*). Second, the resistance in the two aberrant crosses involved resistance of the seeds (i.e. beans) instead of resistance of the vegetative tissue of the plant that was the subject of all other studies. Third, while in all other studies resistance was measured by some index of the relative amount of infection of the host plant, in these two crosses it was quantified by measuring the time until the adult weevil came out of the beans. No skewness was observed for resistance against potato virus Y(1,2) in *Capsicum* and or resistance against *Gibberella zeae* in *Zea mays*.

Table 1. *The nature of epistasis between loci affecting fitness characters in fungi and plants*

Fitness component	Species	F	*n*	Skewness[a] log-scale	Skewness untransformed	h^{2b}_{B}	Reference
Haploids							
Mycelial growth rate	*Aspergillus nidulans*	F_1	98	+1·39	+1·80	0·45	Jinks *et al.* (1966)
		F_1	60	+2·51*	*+3·84***	0·83	Jinks *et al.* (1966)
		F_1	96	*+3·36***	*+4·30***		Caten (1979)[c]
	Schizophyllum	F_1	95	−0·85	+0·18	0·93	Simchen (1966)
		F_1	100	+0·32	+1·59	0·85	Simchen (1966)
	Collybia velutipes	F_1	87	−3·19**	+1·06	0·94	Croft & Simchen (1965)
		F_1	76	−0·18	+0·62	0·90	Croft & Simchen (1965)
		F_1	76	−3·17**	−1·90	0·94	Croft & Simchen (1965)
		F_1	68	−2·60*	−0·85	0·96	Croft & Simchen (1965)
		F_1	95	−1·80	+1·47	0·83	Croft & Simchen (1965)
	Neurospora crassa	F_1	84	−1·99*	−1·54	0·87	Papa *et al.* (1967)
Diploids							
Earliness (flowering time)	*Hordeum vulgare*	DH[d]	69	*−4·30***	−0·09	0·98	Laurie *et al.* (1995)
Earliness (flowering time)	*Orya sativa*	F_8	194	*−9·13***	*−7·62***	> 0·61	Xiao *et al.* (1996)
Earliness (age at maturity)		F_8	194	*−7·68***	*−7·18***	> 0·74	Xiao *et al.* (1996)
Earliness (flowering time)		F_4	2418	*−13·9***	*−10·2***	> 0·77	Li *et al.* (1995*a*)
Earliness (flowering time)	*Arabidopsis thaliana*	F_8	64	*−4·63***	−3·20***	0·96	C. Alonso Blanco[e]
Earliness (flowering time)		F_3	50	*−7·57***	−2·33*		Clarke *et al.* (1995)
Earliness (flowering time index)	*Brassica oleracea*	F_3	92	−0·71	+0·78	> 0·54	Camargo & Osborn (1996)
Earliness (spring bud flush)	*Populus*	F_2	55	−2·84**	−2·05*	0·98	Bradshaw & Stettler (1995)
Resistance to *Pyrenophora teres*	*Hordeum vulgare*	DH	150	*−13·9***	*−6·78***	0·92	Steffenson *et al.* (1996)
Resistance to *Cochliobolus sativus*		DH	150	*−5·55***	−0·98	0·91	Steffenson *et al.* (1996)
Resistance to potato virus Y (1,2)	*Capsicum annuum*	DH	94	−0·01	+1·20	0·90	Caranta & Palloix (1996)
Resistance to *Pyricularia oryzae*	*Oryza sativa*	F_7	281		*−3·26***	> 0·76	Wang *et al.* (1994)
Resistance to *Rhizoctonia solani*		F_4	255	*−5·53***	−1·88	> 0·70	Li *et al.* (1995*b*)
Resistance to *Heterodera glycines*	*Glycine max*	$F_{6:7}$	298	*−36·7***	*−6·45***	0·97	Webb *et al.* (1995)

Resistance to *Gibberella zeae*	*Zea mays*	F_3	112	+0·30	+2·90**	0·37	Pè *et al.* (1993)
Resistance to *Xanthomonas campestris*	*Phaseolus vulgaris*	F_3	70	*−4·86****	−1·68	> 0·75	Nodari *et al.* (1993)
Resistance to *Acanthoscelides obtectus*		F_2	±100	*+4·64****	*+6·44****		Kornegay & Cardona (1991)
		F_2	±100	*+3·25****	*+5·00****		Kornegay & Cardona (1991)
Resistance to *Verticillium*	*Solanum tuberosum*	F_1	100		*−4·71****		Concibido *et al.* (1994)
		F_1	100		*−12·4****		Concibido *et al.* (1994)
		F_1	100		*−7·69****		Concibido *et al.* (1994)
		F_1	100		*−8·67****		Concibido *et al.* (1994)
		F_1	100		*−2·15**		Concibido *et al.* (1994)
		F_1	100		*−4·71****		Concibido *et al.* (1994)
Max. total seed number (long season)[f]	*Arabidopsis thaliana*	F_8	64	+1·27	+2·47*	0·90	C. Alonso Blanco[e]
Max. total seed number (short season)[f]		F_8	64		+0·07	0·92	C. Alonso Blanco[e]
Grain yield	*Oryza sativa*	F_8	194	*−4·08****	−1·10	> 0·14	Xiao *et al.* (1996)
Relative pollen tube growth at 41 °C	*Zea mays*	F_8	45	−1·10	−0·43	0·64	Frova & Sari-Gorla (1994)
Relative pollen germinability at 41 °C		F_8	45		−1·61	0·68	Frova & Sari-Gorla (1994)
Pollen tube growth rate		F_8	45		−0·76	0·71	Sari-Gorla *et al.* (1992)
Pollen germinability		F_8	45	−2·52*	+0·74	0·77	Sari-Gorla *et al.* (1992)
Flowering synchrony (well watered)[g]		F_3	234	*−5·49****	*−3·34****		Ribaut *et al.* (1996)
Flowering synchrony (drought stress)[g]		F_3	234	*−11·0****	*−8·61****	0·78	Ribaut *et al.* (1996)
Proportion flowering at end of season	*Brassica oleracea*	F_3	92	*−4·73****	*−3·63****		Camargo & Osborn (1996)

[a] Skewness: t_s value of the estimate of skewness statistic g_1, at a log-scale or at the untransformed original scale. Negative values indicate synergism between deleterious alleles, positive values antagonism. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$. Cases with significant skewness after correcting for multiple comparisons with the sequential Bonferroni method (see Methods) are given in italic.

[b] Broad-sense heritability $h_B^2 = V_G/V_{tot}$, where V_G is the genetic variance and V_{tot} is the total phenotypic variance.

[c] Only continuous distribution of the three (at 35 °C).

[d] DH, doubled haploid lines produced from F_1 plants.

[e] Unpublished results

[f] Max. total seed number was extrapolated from a representative fruit by multiplying the seed number of this fruit by fruit number of the main stem and by the number of side branches and shoots containing fruits. Long season, all plants ripened their seeds; short season, maximum total seed number when the slowest plant started seed ripening.

[g] Male and female flowering synchrony expressed as the anthesis–silking interval in days.

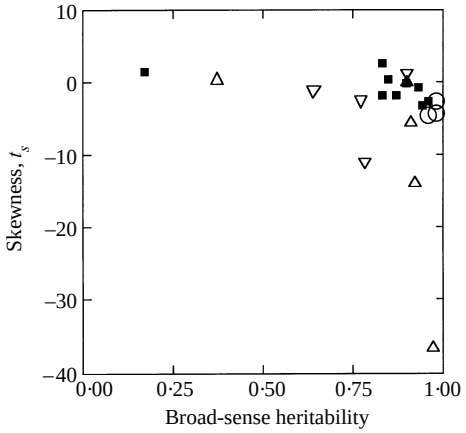

Fig. 1. The effect of error or environmental variance on the skewness of log offspring fitness. Given is the skewness of log offspring fitness versus broad-sense heritability for all crosses for which estimates of the heritability were provided. Different categories of fitness components are represented by different symbols (■, Mycelial GR; ○, earliness; △, resistance; ▽, other). The correlation for all data is not significant ($\rho = -0{\cdot}28$, $n = 22$, $P = 0{\cdot}21$).

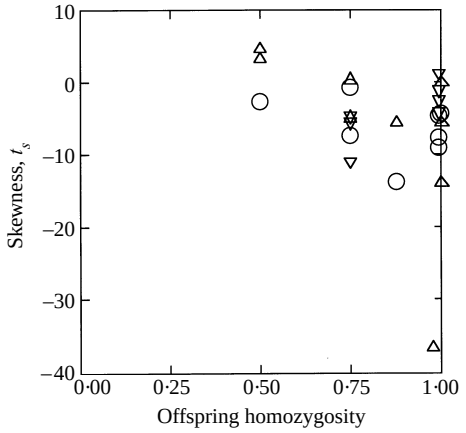

Fig. 2. The effect of dominance on the skewness of log offspring fitness. Given is the skewness of log offspring fitness versus a measure of the amount of homozygosity in the offspring, as derived from the number of generations individual F_1 offspring have been inbred, for all diploid crosses. It has been assumed that parents were fully inbred. Different categories of fitness components are represented by different symbols. The correlation for all data is not significant ($\rho = -0{\cdot}34$, $n = 24$, $P = 0{\cdot}11$).

For the other fitness-related traits, significant skewness of the distributions was only negative, irrespective of the scale of measurement. These characters included grain yield, male and female flowering synchrony and the proportion of all plants of each F_3 family that were still flowering or budding at the end of the season (for a variety of species). Pollen germinability in *Zea mays* showed a nearly significant negative skewness at log scale as well. No significant skewness was observed at this scale for total seed number in *Arabidopsis*, and for other components of pollen fitness in *Zea mays*. However, in three of these five crosses only a value of the skewness of the untransformed data was available, due to the presence of zero values in the data.

(iii) *The effect of error variance*

A skewed error variance (at log scale) may have contributed to the skewness of the distribution of log mean performance per offspring, which may have obscured the skewness caused by epistasis. The relative contribution of the error variance is inversely related to the broad-sense heritability $h_B^2 = V_G/(V_G + V_E)$, where V_G and V_E are the genetic and error variance, respectively. Thus, if the negative skewness found in most crosses would (partly) be due to a negatively skewed error variance, a positive correlation between the skewness, t_s, and h_B^2 is to be expected. Fig. 1 shows that the correlation between t_s and h_B^2 is negative rather than positive if calculated for all data (haploid and diploid) that give an exact estimate of the heritability ($\rho = -0{\cdot}28$, $n = 22$, $P = 0{\cdot}21$; excluding the extreme value of $t_S = -36{\cdot}7$: $\rho = -0{\cdot}31$, $n = 21$, $P = 0{\cdot}18$). Therefore, no evidence for a general contribution of the error variance to the negative skewness observed was found in our data. If skewed at all, the error variance seems to be rather positively skewed, thereby partly masking the negative skewness due to epistasis. Sample sizes were too small to test the relationship between relative error variance and skewness for the various fitness components individually.

(iv) *The effect of dominance*

Dominance, causing the fitness of heterozygotes to deviate from the (log) mean value of both homozygotes, might have affected the skewness of the offspring distributions in the early generations (F_1 and F_2) as well. However, since skewness was significantly negative in the homozygous F_8 and doubled-haploid progenies as well, dominance was clearly not the only cause of skewness. The relative contribution of dominance to the skewness may be revealed by comparing the skewness of progenies with different mean levels of homozygosity. If the negative skewness found were partly due to dominance, one would expect the negative skewness to decrease, or even disappear, with increasing homozygosity of the progeny. Fig. 2 shows the correlation between skewness, t_S, and the level of homozygosity of the progenies. Homozygosity has been derived from information on the number of generations the different recombinant inbred lines have been selfed. It has been assumed that the parents were homozygous and that the F_1 was

completely heterozygous. Doubled-haploid progenies were assumed to be completely homozygous. If the various fitness-related traits were lumped for all diploids, a non-significant negative correlation between skewness and homozygosity was found ($\rho = -0{\cdot}34$, $n = 24$, $P = 0{\cdot}11$). Therefore, if anything, dominance of deleterious alleles causes positive skewness in the early generations (F_1 and F_2), and the increase in negative skewness with increasing homozygosity is likely to be due to synergistic epistasis between segregating deleterious alleles. Again, for individual fitness-related traits sample sizes were too small to test this relationship.

4. Discussion

In this paper we presented the results of a literature search on the nature of epistasis between loci affecting fitness by using information on the skewness of distributions of fitness-related traits among full-sib offspring (De Visser *et al*., 1997*a*). A broad variety of characters was encountered in a restricted number of organisms: fungi and plants. The results for the fitness traits encountered for fungal species were ambiguous, but for plants negative skewness, suggesting synergistic epistasis between deleterious or antagonistic epistasis between beneficial alleles, was found to be common for most fitness-related traits encountered.

(i) *Skewness and synergistic epistasis*

Our conclusions depend on the assumption that the skewness of the log fitness distribution of full-sib offspring is due to epistasis between loci affecting fitness (De Visser *et al*., 1997*a*). However, epistasis is not the only possible cause of skewness. We will discuss the relative importance of three alternative sources of skewness.

In the first place, a negatively skewed error variance due to a skewed distribution of some environmental quality or measurement error might have contributed to the negative skewness that was observed for most fitness traits. Since we considered skewness of the log fitness distribution, a normally distributed error could also have contributed to the negative skewness at log scale (Sinnot, 1937). However, the error variance should be high relative to the genetic variance to make a significant contribution to the skewness. We used our data to check the relationship between the relative contribution of the error variance and the level of skewness, but found no evidence for a positive correlation with negative skewness. However, our analysis was based on the simultaneous evaluation of the error variances of different fitness components, since we could not accurately measure the relative contribution of the error for individual fitness components. It is conceivable that the error may be skewed differently for different fitness components. The studies on the mycelial growth rate in *Collybia velutipes* (Croft & Simchen, 1965) and *Neurospora crassa* (Papa *et al*., 1967) provided distributions of the parental performance as well, which showed a tendency to be negatively skewed. Croft & Simchen (1965) argued that deleterious mutations that occurred during cloning the parental strains may be the reason for this pattern. In the plant studies, no information on the error variance was given, although the application of ANOVA in many studies might suggest that the error variance was roughly normal. Furthermore, the high heritabilities in most studies suggest that the contribution of a possibly skewed error variance was relatively insignificant for most crosses of our review.

Secondly, dominance may have caused skewness in diploid progenies that are heterozygous at many loci (e.g. in the F_2). For instance, if beneficial alleles are dominant, the heterozygote resembles the beneficial homozygote more than the deleterious homozygote, leading to negative skewness. Similarly, recessive beneficial alleles cause positive skewness. If some beneficial alleles are dominant while others are recessive, this may lead to mutual cancelling of the dominance effects, resulting in no skewness. Metabolic arguments exist that support the likelihood of beneficial alleles being dominant (Hoekstra *et al*., 1985). However, for the fitness components in this study, this did not appear to be generally true. Some studies presented the performance of the F_1 and the parents, so that information on dominance could be derived from a comparison between these two generations. This revealed evidence for dominance of earliness alleles in *Populus* (Bradshaw & Stettler, 1995), while in *Arabidopsis* (Clark *et al*., 1995) and *Brassica* (Camargo & Osborn, 1996) alleles were mainly additive. Resistance alleles were found to be recessive in *Phaseolus*, both for resistance against the bean weevil (Kornegay & Cardona, 1991) and against common bacterial blight (Nodari *et al*., 1993), and in *Capsicum* (Caranta & Palloix, 1996), but dominant in *Oryza* (Wang *et al*., 1994), and both dominant and recessive in *Glycine* (Webb *et al*., 1995). However, dominance of earliness alleles did not affect the sign of skewness in the studies we reported, and therefore was at least not a very important factor. For resistance, two studies involving recessive resistance alleles showed positive skewness (Kornegay & Cardona, 1991; Caranta & Palloix, 1996), possibly indicating some dominance effect, but a third study involving recessive resistance alleles showed negative skewness (Nodari *et al*., 1993). Moreover, we tested the effect of dominance on skewness by comparing the skewness between different levels of homozygosity for all traits together, and found a (non-significant) negative correlation between the level of homozygosity and

skewness. This suggests that a possible overall contribution of dominance would cause positive rather than negative skewness, resulting from dominance of deleterious alleles. Thus, it is likely that the increase in negative skewness with increasing homozygosity is caused by synergistic epistasis that is increasingly revealed by the disappearance of obscuring heterozygosity.

In the third place, skewness may be caused by selection of beneficial alleles during the repeated inbreeding of F_1 lines. At each generation of inbreeding, offspring that carry a higher than average number of beneficial alleles for the trait under study might have a higher than average probability of being selected as parents for the next generation. This would lead to negative skewness in the later generations. A special case of such selection bias could be 'segregation distortion', i.e. deviation from the expected Mendelian segregation ratio due to competition among gametes or abortion of the gamete or zygote (Harushima *et al*., 1996). The result is a typical over-representation of a certain part of one parental genome in the offspring (e.g. Wang *et al*., 1994; Xiao *et al*., 1996). Segregation distortion favours genes with high fitness in the gametophyte and the young zygote, but does not necessary affect genes that control fitness components later in life. Its effect on most fitness components in our study may, therefore, be limited. However, it cannot be ruled out that favourable alleles for traits such as pollen fitness or grain yield have increased in frequency during several generations of inbreeding.

In general, negatively skewed log fitness distributions provide conservative support for synergistic epistasis between deleterious alleles. Selection tends to cull individuals in the low-fitness tail of the distribution, causing a shift towards positive skewness. This is illustrated by the observation that in dense monocultures of plants a typical change occurs from a normal distribution of seedling masses to a positively skewed distribution of adult plant masses. This phenomenon has been ascribed to competitive elimination of small individuals (White & Harper, 1970; Ford, 1975; Bazzaz & Harper, 1976), although in grasses this change in the form of the distribution may be rather due to differential growth rates (Turner & Rabinowitz, 1983). Individuals that have been eliminated should actually be given a fitness of zero, but normally these individuals will be missed. Measuring fitness in a laboratory environment may increase the chances of finding negative skewness, since selection will often be limited under such benign conditions.

(ii) *Epistasis for total fitness*

For the epistasis observed in the various fitness-related traits to be relevant for fitness itself, fitness needs to be a linear function of the fitness-related trait. The high heritabilities reported for most traits might cast some doubt on how related these traits are to fitness (Gustafsson, 1986; Burt, 1995). However, the heritabilities reported are broad-sense and not narrow-sense heritabilities, which are more relevant for the evolution of fitness (Burt, 1995) and may be much smaller than the ones reported. One reason for the high heritability values could be that in the crosses studied alleles with large effect were segregating, which improved the detection of genetic factors relative to error variance, and consequently the estimated broad-sense heritability.

Since non-linear relationships between fitness-related trait and fitness change the skewness of the resulting fitness distribution, deviations from linearity may affect our conclusions. One such deviation from linearity that is likely to occur is an optimum relationship between fitness-related trait and fitness, caused by trade-offs between individual characters. There is empirical support for the significance of such trade-offs (e.g. Stearns, 1992; Shook *et al*., 1996; Rausher, 1996). If the optimum curve has an overall concave shape, its second derivative is negative everywhere, which can be shown analytically to cause a negatively skewed distribution of total fitness if the distribution of the fitness-related trait were Gaussian (De Visser *et al*., 1997*a*). Thus, even finding no epistasis at the level of the fitness-related trait may lead to synergistic epistasis between deleterious and antagonistic epistasis between beneficial alleles at the level of total fitness under optimum relationships. The effect of mapping an already negatively skewed distribution on an optimum curve has not been considered, but it seems likely that this will also increase the negative skew. A saturation curve relating fitness-related trait and fitness will increase the negative skewness at the level of fitness as well, because its second derivative is negative too. Only an exponentially increasing relationship may affect our conclusions qualitatively. Therefore, most deviations from a linear relationship between fitness-related traits and fitness do not affect our conclusion that synergistic epistasis between deleterious alleles or antagonistic epistasis between beneficial alleles is rather common in plants.

(iii) *Relevance for the evolution of sex*

The results of this study suggest a rather general existence of synergistic epistasis between deleterious alleles or antagonistic epistasis between beneficial alleles in plants, which is general support for the currently popular theories on the evolution of sex and recombination. However, on the basis of these results we cannot distinguish between the Mutational Deter-

ministic hypothesis, relying on synergistic epistasis between slightly deleterious mutations, and the Environmental Deterministic hypothesis, relying on antagonistic epistasis between beneficial mutations. Distinguishing between the two classes of models depends on the relative importance of the selection pressures caused by recurring deleterious mutations and fluctuating environments, either biotic or abiotic, causing 'narrowing' selection (Shnol & Kondrashov, 1993; Kondrashov & Yampolsky, 1996).

The Mutational Deterministic hypothesis (Kondrashov, 1988) requires synergistic epistasis between slightly deleterious mutations, which are thought to be more relevant for evolution than deleterious mutations with large effect, due to their presumed higher frequency (Kondrashov, 1988; Kobota & Lynch, 1996; but see Peck & Eyre-Walker, 1997). In at least some studies, loci with relatively large effect were involved, explaining up to 50% of the phenotypic variation, which enhanced the detectability of skewness (see De Visser *et al*., 1997*a*). Indeed, the high values of negative skewness observed seem only consistent with a combination of strong epistasis and segregating alleles with a relatively large effect, and not with low or moderate synergistic epistasis between slightly deleterious mutations (see Charlesworth & Barton, 1996, for a related argument applied to the decrease in *mean* fitness caused by sex).

How relevant are our results for epistasis between slightly deleterious mutations? Obviously, our conclusions are directly relevant to slightly deleterious mutations that affect the loci with major effect for which we found synergistic epistasis. To what extent mutations affecting other loci will show synergistic epistasis for fitness cannot be answered directly. However, the finding of synergism between deleterious alleles affecting such various components of fitness, suggests that synergism may be due to very general physiological or metabolic mechanisms. Furthermore, the results obtained for major loci in this study are consistent with the few empirical studies on epistasis between deleterious mutations with smaller effect. In an earlier study we found no evidence for epistasis between marker mutations with relatively small effect that affect the mycelial growth rate of *Aspergillus niger* (De Visser *et al*., 1997*b*), which is consistent with the ambiguous results obtained in this study for the same parameter in a number of fungal species. Willis (1993) studied epistasis between deleterious mutations affecting a number of fitness components in the monkey flower, *Mimulus guttatus*, by comparing different levels of mutation expression due to inbreeding. He found evidence of synergism between slightly deleterious mutations only for pollen viability, which is consistent with our finding of synergism for pollen germinability in maize. However, we cannot check his failure to find synergism for seed germination, flowering and flower production, because these fitness components are not included in our study.

The finding of antagonistic interaction between plant resistance alleles is direct support for the hypothesis that sex has evolved as a means to resist parasites (Jaenike, 1978; Hamilton, 1980). The preservation of resistance alleles, which is essential to the latter hypothesis, is largely enhanced by soft selection (Hamilton *et al*., 1990). Other support for the parasite hypothesis comes from the finding of polygenic rather than monogenic ('gene-for gene') control of resistance in all instances cited in this study. Polygenic control of resistance has been shown to enhance selection for sex and recombination in the light of resisting parasites (Hamilton *et al*., 1990).

There are theoretical arguments for the type of epistasis observed in this study. One argument is that traits with a high impact on fitness are expected to be canalized, i.e. under the control of mechanisms that constrain the trait to be closer to the optimum (Rendel, 1967). Canalization can be against environmental perturbations (e.g. changes in temperature) or against genetic perturbations (e.g. mutation). Genetic canalization has been demonstrated to increase with a trait's impact on fitness in *Drosophila melanogaster* (Stearns & Kawecki, 1994). If canalization were perfect up to a certain mutation load, this would result in truncation selection against mutations, i.e. no effect of mutation accumulation on fitness up to a certain mutation number, beyond which fitness decreases steeply. Less perfect canalization may lead to more moderate synergistic mutation selection. That the phenotypic suppression of mutational damage to developmental homeostasis may be limited up to a certain mutation load has been hypothesized by Kimura & Maruyama (1966). Alternatively, the presumed existence of truncation-like selection in situations of high population density may cause the same type of epistasis (Crow, 1988; Hamilton *et al*., 1990). If fitness is dependent on contesting ability and the latter depends on either mutation load or parasite resistance, this will cause both synergism between deleterious mutations and antagonism between beneficial, i.e. resistance, alleles (Hamilton *et al*., 1990). This ecological argument seems compelling to us, and might explain the prevalence of sexual reproduction in saturated environments (Bell, 1982).

To summarize, we have provided empirical evidence for a fairly general occurrence of synergistic epistasis between deleterious alleles or antagonistic epistasis between beneficial alleles in a variety of plant species. These results lend substantial support to the currently popular models of the evolution of sex and recombination (Barton, 1995; Charlesworth & Barton, 1996), but they do not distinguish between them. For that purpose, knowledge on the relative selection

pressures caused by recurrent deleterious mutations and fluctuating environments is needed.

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