The Expression of Additive and Nonadditive Genetic Variation Under Stress

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ABSTRACT

Experimental lines of *Drosophila melanogaster* derived from a natural population, which had been isolated in the laboratory for ~70 generations, were crossed to determine if the expression of additive, dominance and epistatic genetic variation in development time and viability was associated with the environment. No association was found between the level of additive genetic effects and environmental value for either trait, but nonadditive genetic effects increased at both extremes of the environmental range for development time. The expression of high levels of dominance and epistatic genetic variation at environmental extremes may be a general expectation for some traits. The disruption of the epistatic gene complexes in the parental lines resulted in hybrid breakdown toward faster development and there was some indication of hybrid breakdown toward higher viability. A combination of genetic drift and natural selection had therefore resulted in different epistatic gene complexes being selected after ~70 generations from a common genetic base. After crossing, the hybrid populations were observed for 10 generations. Epistasis contributed on average 12 hr in development time. Fluctuating asymmetry in sternopleural bristle number also evolved in the hybrid populations, decreasing by >18% in the first seven generations after hybridization.

IT is well known that the environment will influence the level of additive genetic variance, and in particular, an increase in environmental stress may change the additive genetic variance in a predictable fashion (LANGRIDGE 1963; PARSONS 1983). An increase in environmental stress has been associated with an increase in heritability in many cases, although the opposite trend has also been found (reviewed in HOFFMANN and PARSONS 1991). In addition to the environment influencing the level of additive genetic variance, the levels of dominance and epistatic genetic variance may also be influenced by the environment. Although heterosis appears to increase with stress levels, no clear trend is apparent for the effects of environmental stress on dominance genetic variance when directly measured (HOFFMANN and PARSONS 1991). An increase in epistasis in a number of traits at environmental extremes was found in crosses between varieties of *Nicotiana rustica* (JINKS *et al.* 1973), the only study to directly address this issue. HOFFMANN and PARSONS (1991, p. 126) review a number of reasons why the genetic components of variance may change under stressful conditions.

The primary aim of this study was to determine if simple relationships exist between the expression of the additive, dominance and epistatic genetic variation and stress. The genetic components of two fitness-related traits, development time and viability, were estimated from crosses between *Drosophila melanogaster* lines that had been founded from a single field population and then isolated in the laboratory for 4 years. Five replicate crosses between different lines were carried out and the generations required to estimate the various genetic effects were cultured under three environmental conditions.

The lines crossed in this experiment were found to exhibit significant levels of epistatic genetic variation for both traits investigated. It has long been recognized that when different geographic populations of the same species are crossed, hybrid breakdown (sometimes called F_2 breakdown or outbreeding depression) may occur in the subsequent backcross and F_2 generations (sensu VETUKHIV 1953; WALLACE and VETUKHIV 1955; reviews in ENDLER 1977; WRIGHT 1977). This phenomenon has been attributed to coadaptation, or integration, of the respective gene pools of each population. In quantitative genetic terms, epistatic genetic variation contributes a significant proportion of the level of the trait under investigation. Therefore, the experimental design in this study allowed a test of the ability of drift and uniform selection to produce epistatic genetic variation between lines derived from a common genetic base. The generation of epistatic genetic variation between replicate lines from a single population has been previously investigated by imposing directional selection for a trait and then determining the level of epistasis in the response. Significant levels of epistasis were demonstrated in some cases (KING 1955; ENFIELD 1977; COHAN 1984). In the present instance, natural selection under laboratory conditions has replaced the directional selection regimes found in these studies.

A further aim of this study was to monitor the evolu-

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tion of development time and viability over a number of generations after hybridization to investigate the behaviour of these two traits after the disruption of the epistatic gene complexes. This situation is analogous to phase III of Wright's shifting balance theory of evolution, where one adaptive peak invades another, with the exception that migration between the two peaks has occurred all at once rather than at a low level. The hybird populations were allowed to evolve under the same rearing conditions, which had persisted for the previous 4 years, for 10 generations.

MATERIALS AND METHODS

Stocks: The *D. melanogaster* lines used were created by one of us (M.B.S.) from a collection of 500 adult flies from an orchard near Toronto, Canada, in October 1988. After being kept in the laboratory for 1 year at 20° in 60 culture bottles each generation, six lines were founded from the progeny of single males. The second chromosomes of these lines were then placed on a heterogeneous genetic background by the use of a chromosome-*2* balancer stock that had been backcrossed to the orchard population for 10 generations (PEREIRA and SOKOLOWSKI 1993). This was accomplished as follows: single males were crossed to a multiply-marked balancer strain *In(1)FM7, y31d sc8 wa B;Pu2/ In(2LR)SM5, al2 Cy lyv sn2 sp2l Ly/ In(3LR)TM3, y+ ri pp sep l(3)89Aa bx43e e* (described in LINDSLEY and ZIMM 1992) to isogenize the second pair of chromosomes thus creating iso-2 lines. During this process the chromosomes carrying the balancers were removed so that no genetic variation from the original balancer strain was left in these iso-2 male lines. Each iso-2 male line represented an independent sample of an intact pair of second chromosomes from nature that comprise 40% of the genome of this species (ASHBURNER 1989). Each iso-2 line was then crossed to a heterogenous orchard-derived genetic background. This was done by crossing each line to a chromosome-*2* balancer strain *In(2LR)SM1, al2 Cy cn2 sp2/In(2LR)bwv1, ds33k bwv1* (described in LINDSLEY and ZIMM 1992), which had been previously backcrossed each generation to a large sample (2000) of flies from the orchard population for 10 generations. Once again, the chromosomes carrying the balancers were removed in the crossing procedure so that little to none of the genetic background from the balanacer strain was left in the resultant lines. Thus each of the lines used in this study had a pair of second chromosomes sampled from nature, while the other chromosomes (*X, Y, 3* and *4*) that comprise the remaining 60% of the genome were as variable as those found in the orchard population. The lines are designated throughout this study as R70.1, R30.2, R70.3, S15.3, S15.4 and S80.1 and were maintained at 20° in two culture bottles/line for the next 4 years (~70 generations). All the experiments described below were conducted at 20°.

Epistatic genetic variation in parental lines: Five replicate crosses were made between the six lines; R70.3 × S15.3, S80.1 × R30.2, R70.1 × S15.4, S80.1 × S15.3 and R30.2 × R70.3. These five crosses were set up in a reciprocal fashion with 25 females and 25 males in each cross. The crosses needed to estimate the genetic effects in a digenic model (parental, reciprocal F_1s, F_2 and both backcrosses) were generated using 60 females and 20 males for each cross. Development time and viability were the two fitness measures made. Development time to eclosion was scored in 12-hr intervals, and the number of emerging adults was used as the score of viability. The procedure was as follows: the 60 females of each cross were allowed to mate for 7 days and then allowed to lay on a cup containing a molasses/cream of wheat medium for 20 hr. The cups were cleared of any larvae that had hatched and were then left for a further 4 hr. The larvae that had hatched after this 4-hr period were used in the experiments.

Associations between genetic parameters and stress: The development time and viability measures were made in three different environments. The first environment consisted of 10 larvae from a cup and 10 larvae of the same age from a *D. melanogaster* stock marked with the mutation *white* (*w*) placed in a standard vial containing 10 ml of a low nutrition medium. This medium had only 5% of the amount of yeast normally used to maintain the stocks. This treatment will be referred to as "low food". Seven replicate vials for each cup were set up. The second environment was standard medium ("high food/propionic acid") and the third was standard medium without the addition of propionic acid which is used as an antibacterial agent in the standard medium ("high food"). Five larvae from a cup were placed into vials containing these two media, with 14 replicate vials set up for each cross for both of these environments. The three environments tested were not set up concurrently in a single block design, but were tested on different days. Therefore, when testing for the presence of differences between the environments below, a possible day effect is confounded with the environment term. The vials from each of the three environments were placed in their own complete randomized block designs.

Evolution of hybrid populations: To determine how natural selection would affect the hybrid populations in subsequent generations, the F_1 and F_2 populations were turned over to initiate F_2 and F_3 generations, respectively, and F_1 crosses were again set up from the parental lines. This procedure was continued until an F_{10} generation had been reached and allowed all generations to be tested in the same experiment. Each generation from the five replicate crosses was maintained in one culture bottle at a population size of >200 individuals. At the time when the F_7 generation had been reached, the development time and viability of the parents and F_1–F_7 generations were measured (referred to as the "F_7 experiment"). A further experiment measuring the development time and viability of the parents and F_2–F_{10} generations followed ("F_{10} experiment"). Both experiments were conducted in the high food/propionic acid environment in complete randomized block designs. Ten vials containing five larvae were set up for each generation of the five replicate crosses for both experiments.

In addition to development time and viability, fluctuating asymmetry (FA) of sternopleural bristle number was measured on the emerging flies in the F_7 experiment. FA was scored as the absolute difference in bristle number between the right- and left-hand sides.

RESULTS

Means and standard deviations for development time and viability for the parental, F_1, F_2 and backcross generations in the three environments are found in Figures 1 and 2, respectively. The graph for development time in low food for the cross R70.1 × S15.3 is not shown because no individuals of the parental line S15.3 completed development to eclosion. Development in the low food environment proceeded much slower and spanned a far greater length of time than in the other two environments; eclosion began after 17 days and spanned over 20 days from the first to last individual compared to the other two environments in which indi-

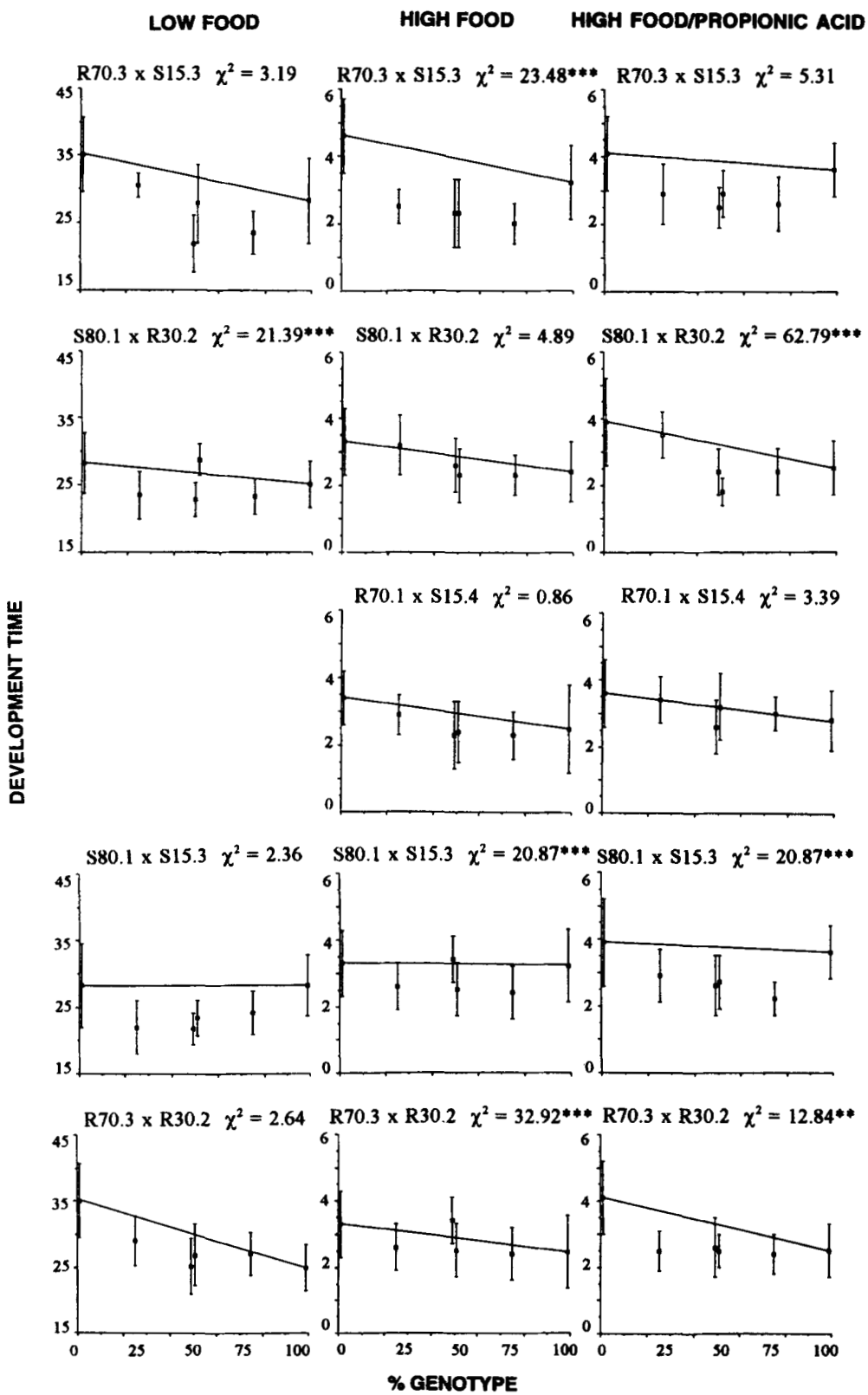

FIGURE 1.—Generation means and single standard deviations in development time for the five replicate crosses in three environments. Development time was measured in 12-hr intervals from the begining of eclosion. The values for the F_1 and F_2 generations are slightly offset over the 50% genotype mark for clarity (the F_1 value appears to the left on all graphs). Chi-square values, with three degrees of freedom, are presented for the joint scaling tests for each cross in each environment.

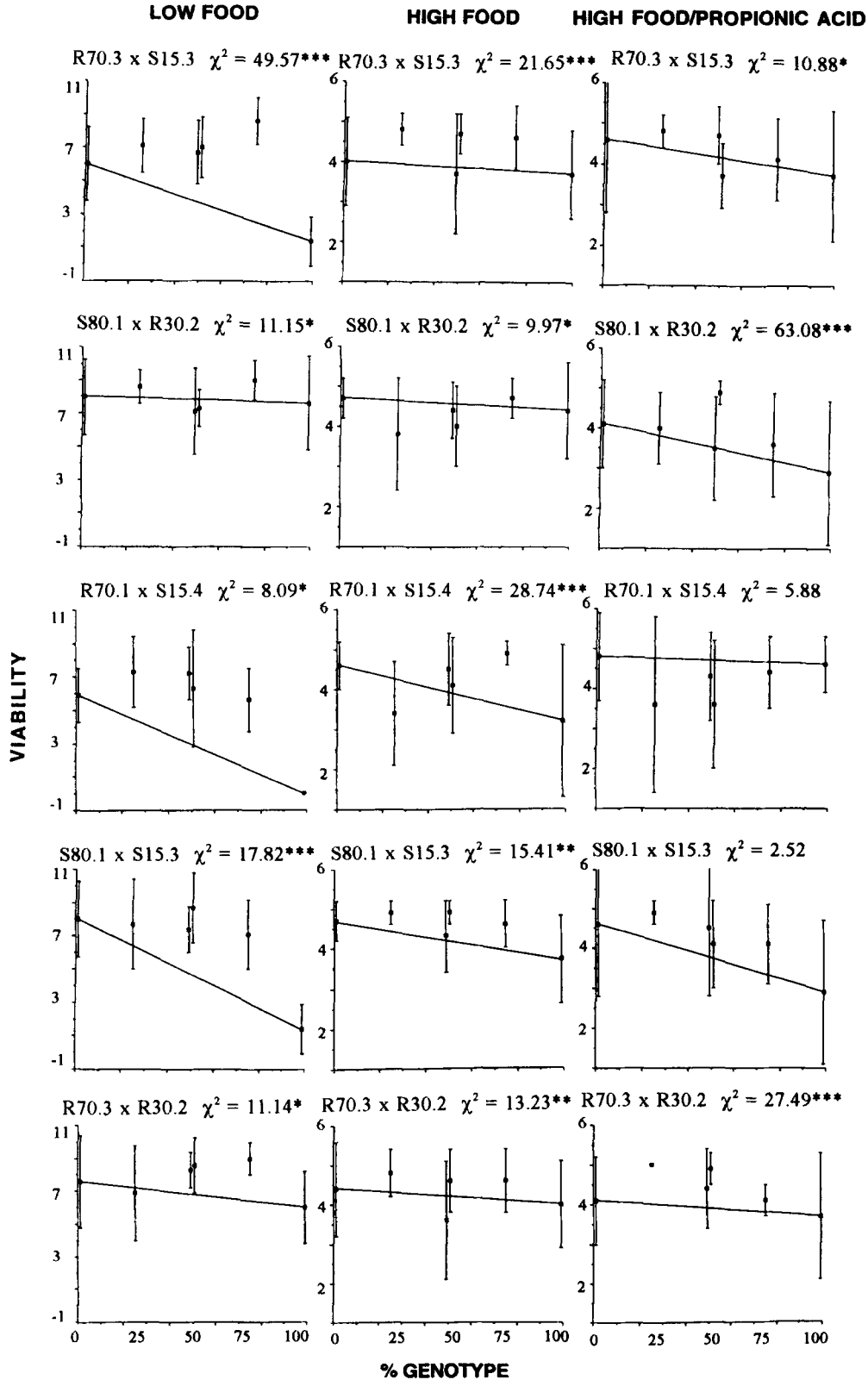

FIGURE 2.—Generation means and single standard deviations in viability for the five replicate crosses in three environments. Viability was measured as the number of individuals surviving to eclosion out of 10 for the low food environment and the number of individuals surviving to eclosion out of five in the other two environments. The values for the F_1 and F_2 generations are slightly offset over the 50% genotype mark for clarity (the F_1 value appears to the left on all graphs). Chi-square values, with three degrees of freedom, are presented for the joint scaling tests for each cross in each environment.

viduals began eclosion after 11 days and spanned just 4 days. Mean viability across all crosses was 72% in the low food environment, 87% in high food and 86% in high food/propionic acid.

Epistatic genetic variation in parental lines: The presence of epistasis was tested using CAVALLI's (1952) joint scaling test, which tests for a significant deviation from the expectations of the additive-dominance model. The chi-square values from these tests (with three degrees of freedom) for development time and viability are found in Figures 1 and 2, respectively. The probability values for these tests were corrected for the number of multiple comparisons by applying the sequential Bonferroni technique (see RICE 1989) across the tests for each trait in each environment (*i.e.*, groups of five tests). All tests remain significant with the exception of two viability tests; R70.1 × S15.4 in low food and S80.1 × R30.2 in high food/propionic acid.

The joint scaling tests indicate the presence of epistasis for 11 out of the 15 crosses for viability and seven out of the 14 crosses display a significant level of epistasis for development time. The segregating generations are generally faster in development than expected under the additive dominance model and at the same time there is some indication of increased viability.

The joint scaling tests do not change appreciably after using the $\log_{10}$ transformation. The choice of scale in biometrical genetics has usually been determined by a desire to simplify the data and make it conform to the additive-dominance model so greater precision of predictions based on the additive component is realized (FALCONER 1981; MATHER and JINKS 1982). However, there is no biological reason why one scale better represents the genetical situation than another, and it is therefore justifiable to choose the scale that maximizes those components of interest (MATHER and JINKS 1982, p. 365). Because the epistatic components are of direct interest in the present context, there seems little point in searching for a more extreme transformation to eliminate these nonadditive effects.

Estimates of the additive, dominance and interaction parameters for development time and viability are displayed in Tables 1 and 2, respectively. The six parameters under the model incorporating digenic epistatic interactions are; m, the mean; $[d]$, the additive component; $[h]$, the dominance component; $[i]$ the additive × additive interactions; $[j]$, the additive × dominance interactions; and $[l]$, the dominance × dominance interactions. Although the epistatic parameters are not usually estimated when the joint scaling test indicates the additive/dominance model is sufficient to account for the variation present, they are presented in this instance because the estimates of these parameters were used in further analyses below (following JINKS *et al.* 1973). Significance for each parameter was retested using the sequential Bonferroni technique across each of the six parameters for each trait (*i.e.*, groups of 14 tests for development time and 15 tests for viability).

There is only sporadic significance across all crosses and environments for each parameter. There appears to be some indication of dominance for genes causing faster development (*i.e.*, $[h] < 0$) in most cases, although the estimate for S80.1 × R30.2 in high food/propionic acid is positive and highly significant and two values do not remain significant after the Bonferroni correction. The direction of dominance of genes contributing to viability is generally toward higher viability, but the estimate for S80.1 × R30.2 is once again significant in the opposing direction and all values lose significance after correction for multiple comparisons.

The loss of fitness in the F_2 relative to that of the midparent will be a consequence of the loss of favorable $[i]$ and $[l]$ effects (HILL 1982; MATHER and JINKS 1982; LYNCH 1991). However, the epistatic parameters need to be interpreted with caution because the signs of $[i]$ and $[j]$ do not necessarily reflect the direction of interactions between individual pairs of genes or even the majority of genes (MATHER and JINKS 1982). This is because these estimates are not independent of the degree of association (*i.e.*, the proportion of genes of increasing effect in each of the parental strains) and therefore the classification of epistatic interactions, in the absence of such information, relies upon the magnitude and sign of $[h]$ and $[l]$. Many estimates of the parameters $[i]$, $[j]$ and $[l]$ in Tables 1 and 2 are not significant. Some estimates of dominance × dominance epistasis for development time are positive and significant indicating interactions in the direction of faster development (*i.e.*, interactions in the parental lines produced slower development), although all but one value lose significance after correction for multiple comparisons. Once again, the estimate for the cross S80.1 × R30.2 in high food/propionic acid is highly significant but in the other direction. The significant values of $[l]$ for viability are negative, as are most of the nonsignificant values, indicating interactions in the direction of higher viability (interactions in the parental lines produced lower viability). For both traits, the signs of $[h]$ and $[l]$ are predominantly in opposing directions which suggests that the interactions are generally of a duplicate type (MATHER and JINKS 1982).

Associations between genetic parameters and stress: To determine if the genetic parameters in Tables 1 and 2 change with the environment (*i.e.*, testing for the presence of genotype × environment interactions), the procedure described in MATHER and JINKS (1982, p.108) for an arbitrary set of environments was followed. To be able to compare estimates across environments, the parameters in Tables 1 and 2 needed to be controlled for differences between environments. This was done by taking the mean of each parameter for each cross (for example, in Table 1 for the cross S15.3 × R70.3, the values 34.90, 4.10 and 4.45 were

TABLE 1

Estimates of the six parameters in the digenic model between the crosses for development time across three environments

Parameter (d.f.)	Cross				
	S15.3 × R70.3	S80.1 × R30.2	S15.4 × R70.1	S15.3 × S80.1	R70.3 × R30.2
m (4)					
L	34.90***	47.80***	—	29.65***	25.65***
H	4.10***	1.05	2.15	3.25*$^{+}$	3.10*$^{+}$
H/P	4.45*$^{+}$	−1.40	3.20	4.35***	3.50***
[*d*] (1)					
L	3.40	1.60	—	0.05	5.05
H	0.70**$^{+}$	0.45	0.45	0.05	1.10***
H/P	0.25	0.70**$^{+}$	0.40	0.15	0.80***
[*h*] (5)					
L	−15.30	−51.70***	—	−17.05	6.05
H	−5.40*$^{+}$	3.45	0.85	−3.15	−3.80
H/P	−4.25	9.00***	0.60	−4.85*$^{+}$	−3.10
[*i*] (2)					
L	3.20	−21.20***	—	−1.40	4.60
H	−0.20	1.80	0.80	0.00	0.40
H/P	−0.60	4.60***	0.00	−0.60	−0.20
[*j*] (3)					
L	7.20	−2.80	—	−4.30	−6.30
H	−0.40	0.90	0.30	0.30	−0.60
H/P	0.10	0.80	0.00	1.10	−1.40*$^{+}$
[*l*] (5)					
L	2.20	26.60**	—	9.10	−6.30
H	3.60*$^{+}$	−1.90	−0.70	3.30*$^{+}$	3.60*$^{+/1}$
H/P	0.30	−5.20***	−1.20	3.10*$^{+}$	2.20*$^{+}$

Significance of each individual estimate was by a *t*-test with the degrees of freedom for each test listed in parenthesis. These probabilities were then reassessed using the sequential Bonferroni technique (see text) and values which lose significance are indicated by +. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. L, low food environment; H, high food environment; H/P, high food/propionic acid environment.

averaged for the parameter *m*) and subtracting the mean from each value. In the terminology of MATHER and JINKS (1982), the new parameters calculated in this fashion from *m*, [*d*], [*h*], [*i*], [*j*] and [*l*] are: e_j, the mean effect of environment *j*; g_{di}, the interaction between [*d*] and environment *j*; g_{hj}, the interaction between [*h*] and environment *j*; g_{ij}, the interaction between [*i*] and environment *j*; g_{jj}, the interaction between [*j*] and environment *j*; and g_{lj}, the interaction between [*l*] and environment *j*, respectively. The cross S15.4 × R70.1 for development time was excluded from this analysis because estimates for the genetic parameters in the low food environment were not available.

Strong parabolic relationships were found between *e* and the absolute values of g_h, g_i and g_l ($0.001 < P < 0.01$, $r^2 = 0.95$; $0.01 < P < 0.02$, $r^2 = 0.93$; $0.01 < P < 0.02$, $r^2 = 0.80$, respectively) for development time and are shown in Figure 3a. No significant relationships were found between *e*, g_d and g_j. The levels of dominance, additive × additive epistasis and dominance × dominance epistasis increase at both extremes of the environmental range measured. No relationships were found between *e* and the other parameters for viability.

Because the levels of g_h and g_l in development time increase at environmental extremes, it is of interest to determine the association between the levels of g_h and g_l. A strong linear relationship between the absolute values of g_h and g_l for development time was found (b = 0.503; $P < 0.001$; $r^2 = 0.92$; Figure 3b). Interestingly, even though no association was found between *e* and g_h or g_l for viability, a similar relationship between g_h and g_l is apparent as for development time (b = 0.592; $P < 0.001$; $r^2 = 0.89$). An increase in the level of dominance results in approximately half the level of that increase in dominance × dominance epistasis. No associations were found between absolute levels of g_i, g_j and g_l between the two traits.

Evolution of hybrid populations: The mean development time, viability and FA of the eight generations from the F_7 experiment, based on the five replicate crosses are shown in Figure 4. FA seems to display heterosis for decreased FA, suggesting that the lines were inbred to some extent. No association was found between the decrease in FA and a decrease in the number of bristles present (MATHER 1953; BRADLEY 1980). No indication of epistasis was found for this trait in any of the five replicate crosses based on scaling test C (MATHER and JINKS 1982) estimated using orthogonal contrasts. To test for the presence of selection on FA, an analysis of covariance was first conducted across the

TABLE 2

Estimates of the six parameters in the digenic model between the crosses for viability across three environments

Parameter (d.f.)	Cross				
	S15.3 × R70.3	S80.1 × R30.2	S15.4 × R70.1	S15.3 × S80.1	R70.3 × R30.2
m (4)					
L	0.30	1.80	2.36	9.90*+	9.40*+
H	3.85***	3.55*+	3.70*+	4.80***	3.80***
H/P	1.15	7.90***	3.10	2.15	5.30***
[*d*] (1)					
L	2.30***	0.20	2.95***	3.30***	0.80
H	0.15	0.15	0.70*+	0.50**+	0.20
H/P	0.45	0.60*+	0.10	0.85	0.20
[*h*] (5)					
L	20.40**+	16.70**+	10.93	−2.30	−2.10
H	3.55	0.95	0.80	0.90	3.40
H/P	6.65*+	−7.60**+	0.80	5.45	−0.70
[*i*] (2)					
L	3.40	6.00**+	0.60	−5.20	−2.60
H	0.00	1.00	0.20	−0.60	0.40
H/P	3.00**+	4.40***	1.60	1.60	−1.40**+
[*j*] (3)					
L	−7.60***	−1.20	−2.49	−5.40	−5.80*+
H	0.10	−2.10*+	−4.40***	−0.40	0.00
H/P	0.50	−0.40	−1.80	−0.10	1.40*+
[*l*] (5)					
L	−14.00**+	−11.40**+	−6.09	−0.20	1.00
H	−3.70**+	−0.10	0.00	−1.40	−3.60*+
H/P	−3.10	3.20	0.40	−3.10	−0.20

Significance determined as in Table 1. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.01$. L, low food environment; H, high food environment; H/P, high food/propionic acid.

data for the five replicate crosses which indicated that the trend across the generations was the same for all five replicates (generation by replicate interaction: $0.1 < P < 0.2$). Next, a regression was carried out using the mean values displayed in Figure 4, with the exception of the F_1 mean because its position is likely to be strongly influenced by heterosis. The significant regression ($b = -0.038$; $P < 0.001$; $r^2 = 0.94$) suggests that natural selection was rapidly decreasing levels of FA in the hybrid populations.

The data for development time in Figure 4 once again suggests heterosis in the direction of decreased development time. More interesting, however, is the continued decrease in development time to the F_6 generation as expected in the presence of two hybridizing adaptive peaks. If the position of the F_2 and subsequent generations was solely a consequence of heterosis, they would be expected to fall half way between the midparent and F_1 in the absence of epistasis (FALCONER 1981). The mean difference between the midparent and the F_2 in development time is 7.3 hr, with a further decrease of 4.6 hr from the F_2 to the F_6. The pattern for viability is less clear. The mean parental viability in Figure 4 is low (87%), which is consistent with the data from the initial crosses, although there is no evidence for an average effect of heterosis in the F_1. Viability is higher than parental levels in the F_2–F_5 generations then falls back to parental levels. The mean increase in viability between the parental and the F_5 is 6.8%.

The development time and viability of the parents and F_2–F_{10} generations from the F_{10} experiment are displayed in Figure 5 for each of the five replicate crosses. When considered individually, the variation in the response of the five hybrid populations to the forces of recombination and selection can be seen. In development time, cross S80.1 × S15.3 appears to display the classic response of a decrease and then the beginnings of a return to parental levels. Cross S15.4 × R70.1 decreases to the F_6 then begins to fluctuate with the F_{10} reaching the value of the midparent and the cross S80.1 × R30.2 displays a similar pattern with the exception of a surprisingly slow mean development time for the F_4. The remaining two crosses exhibit little pattern. The data for viability is more ambiguous. The only consistent pattern appears to be higher viability in the initial segregating generations in four of the five crosses.

DISCUSSION

Epistatic genetic variation in parental lines: After ~70 generations (4 years), the lines used in this experiment seem to have evolved different coadapted complexes as a result of drift from a common genetic base. Early work on the so-called "Vetukhiv populations" of

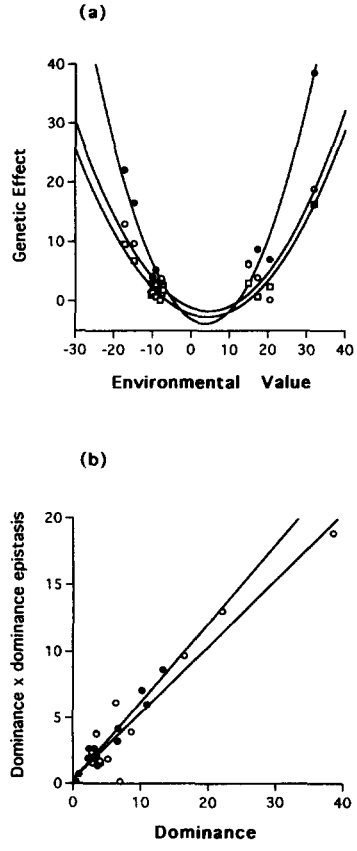

FIGURE 3.—The association between the environmental value and nonadditive genetic variation. (a) Environmental value (e) plotted against the levels of dominance g_h (●), additive × additive epistasis g_i (□) and dominance × dominance epistasis g_l (○) in development time. (b) Level of dominance g_h plotted against the level of dominance x dominanace interaction g_l in development time (○) and viability (●).

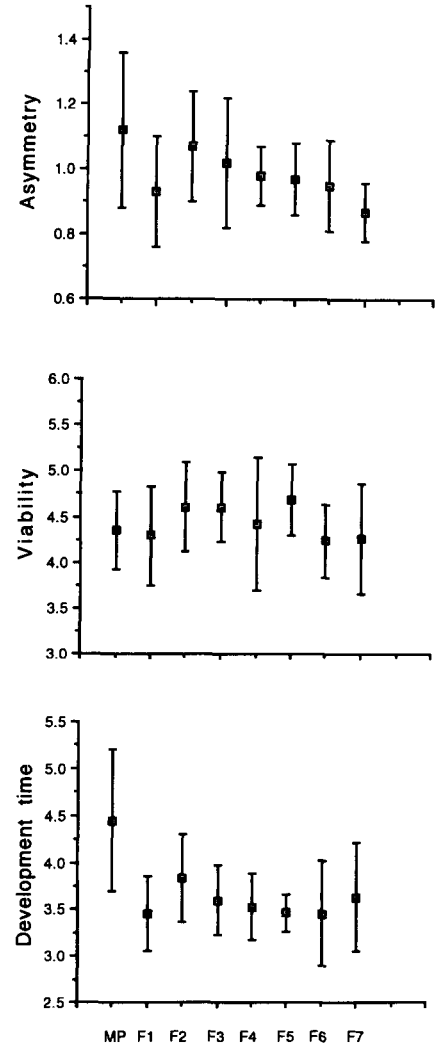

FIGURE 4.—Generation means and single standard deviations for the midparent (MP) and the first seven generations after hybridization. Values for each of the three triats are averaged across the five replicate crosses. Fluctuating asymmetry in sternopleural bristle number was measured as the absolute difference in bristle number between the right and left sides, viability was measured as the number of flies surviving to eclosion out of five and development time is measured in 12-hr intervals from the beginning of eclosion.

D. pseudoobscura, comprising of replicate lines which were maintained at three temperatures, has also indicated that drift between lines may cause different epistatic interactions to evolve. MOURAD (1965) found hybrid breakdown in longevity after 4.5 years between the lines from the same temperature (drift) as well as different temperatures (a combination of selection and drift). KITAGAWA (1967) also reported hybrid breakdown in viability after 8 years between these lines from the same and different temperatures. Unfortunately, neither study made an explicit test of whether more breakdown was generated between lines from different temperatures over that found between lines from the same temperature to determine the relative importance of drift and selection.

The epistatic interactions detected for both traits appear to be predominantly of a duplicate type. Duplicate-type interactions reduce the variance in segregating generations and therefore the number of genotypes of lower fitness and are typical of traits thought to be under directional selection (MATHER 1967, 1973). Directional selection for slower development under laboratory conditions, as appears to be the case in this instance, poses no particular conceptual difficulties and has been implicated in laboratory populations of another Drosophila species (BLOWS 1993). However, the indication that epistatic interactions confer lower viability in the parental lines is more problematical. There are at least two reasons why hybrid breakdown may occur in the direction of what appears to be greater fitness in viability. First, genes that have been in isolation from each other for 4 years may act more beneficially together than those genes that have been in the same gene pool. This represents the neutral or null hypothesis and would be evidence against the existence of coadapted gene complexes, although it seems unlikely that such favorable interactions should occur consistently at random. However, LYNCH (1991) reported an

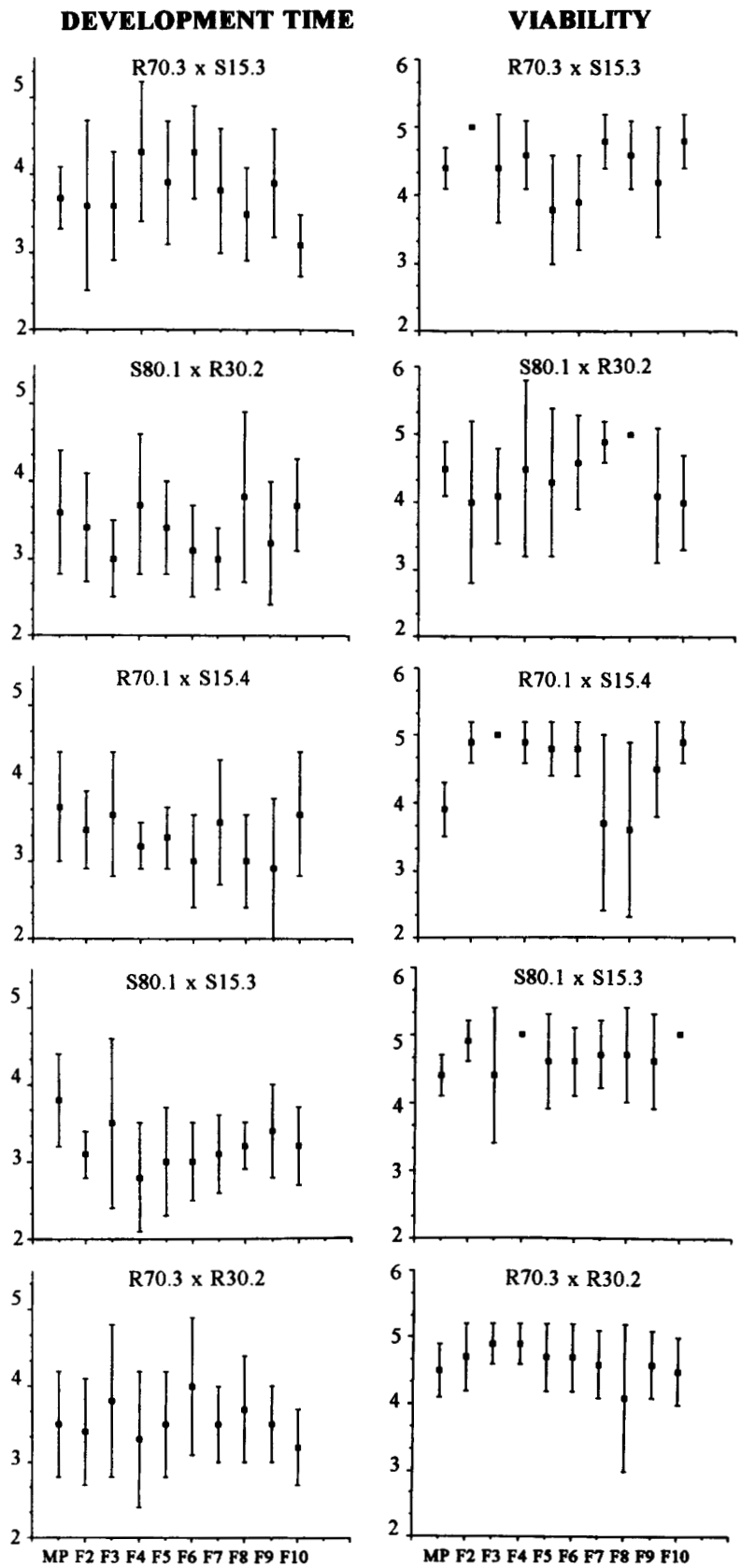

FIGURE 5.—Generation means and single standard deviations for the midparent (MP) and F_2–F_{10} generations after hybridization. Development time and viability are measured as in Figure 4.

increase in grain yield in the F_2 between population crosses of maize after a reanalysis of data from MOLL *et al.* (1965). Second, the increase in viability after the breakup of any coadapted gene complexes may indicate that viability is maintained by selection at a level below the maximum level obtainable. That is, an increase in viability as measured by these experiments may not result in a selective advantage in the laboratory environment (BREESE and MATHER 1960). For instance, there may be selection for some unknown trait and the maintenance of a lower level of viability may represent a cost associated with gaining the required level of that trait. The evolution of interactions for lower viability may then represent an attempt to reduce the impact of that cost. However, the inconsistent nature of the behavior of viability during the first 10 generations after hybridization (Figure 5) precludes any firm conclusions concerning the evolution of viability in these populations.

Associations between genetic parameters and stress: Dominance, additive × additive epistasis and dominance × dominance epistasis in development time increased at both extremes of environmental conditions in a remarkably similar fashion. In general, the evidence for dominance genetic variance increasing under stressful conditions is equivocal (HOFFMANN and PARSONS 1991). In the only other study to directly address the issue of the expression of epistatic genetic variation under stress, JINKS *et al.* (1973) found additive × additive epistasis increased at both extremes of an environmental range for growth rate and leaf length in *Nicotiana rustica*, but increased toward only one extreme for plant height and flowering time. FERRARI (1987) found that levels additive × additive epistasis in development time in *D. melanogaster* males were higher at 17° than more optimal temperatures, although a direct test of the differences between environments was not made. An increase in epistasis at extremes may therefore be a general phenomenon for some traits. If the components of genetic variation do generally change at both extremes of an environmental range, this may obscure relationships between these components and stress when only two environmental values are used to investigate this issue, as has often been the case in the past (HOFFMANN and PARSONS 1991). Therefore, experiments designed to determine the association between stress and genetic variation need to consider a range of environmental values and to distinguish between the various genetic components.

HOFFMANN and PARSONS (1991, p.126) list a number of alternative explanations that may account for changes in genetic components with stress levels. Of these, one specifically addresses the expression of dominance and epistatic genetic variation. The history of selection that a trait has experienced may be reflected in its genetic basis (MATHER 1973). Continued directional selection on a trait would be expected, not only to select for dominant expression of genes in the direction of selection (FISHER 1930), but to select for duplicate-type interactions between genes (MATHER 1967, 1973). A trait under stabilizing selection, on the other hand, tends to exhibit little pattern in the direction of dominance and epistasis is weak. JINKS *et al.* (1973) suggested that the same pattern in epistasis is exhibited when the genetic basis of a trait is investigated under stressful and nonstressful conditions; epistasis becomes increasingly important in the genetic basis of a trait when measured under stressful conditions. Stress is likely to increase the directional nature of selection. Although the development time data conforms well with these expectations, viability does not, in spite of the presence of significant levels of epistasis in this trait.

Although levels of dominance and epistasis in viability were not associated with the environmental value, a similar linear relationship existed between dominance and dominance × dominance epistasis for both traits, suggesting a simple relationship between the levels of these two components of genetic variation. It is unclear at this stage whether there is any significance in both traits exhibiting approximately the same relationship between dominance within loci and the interactions between heterozygotes; an increase in dominance resulting in about half that increase in heterozygote interaction. Indeed, it is not clear if relationships between levels of epistasis and their additive and dominance components are to be generally expected. JINKS *et al.* (1973) did not find a relationship between the levels of the additive component and additive × additive epistasis in the characters height, growth rate, leaf length and flowering time of *N. rustica*, in the presence of significant levels of [*i*].

Evolution of hybrid populations: The new genetic constitutions of the hybrid populations were rapidly acted upon by natural selection. Levels of fluctuating asymmetry in bristle number decreased steadily by >18% in the first seven generations after hybridization. FA has been shown to respond to selection under a variety of circumstances (review in PARSONS 1990). In particular, after the disruption of coadaptation resulting in an increased level of FA, FA may then decrease as new coadapted complexes arise. A significant amount of epistasis was not demonstrated in FA in the present study, although this test was weak because it was based on a single scaling test only. CLARKE and MCKENZIE (1988) showed that resistance to the insecticide diazinon was initially associated with increased levels of FA but returned to susceptible levels as a consequence of the evolution of modifiers by continually backcrossing the resistant phenotype onto the susceptible background and recording an associated increase in FA.

Selection also seems to have begun to return the levels of development time to parental levels, at least in some of the five replicate crosses, by the end of the experiment. Hybrid breakdown between two popula-

tions has been interpreted as evidence for the existence of different adaptive peaks (*e.g.*, COHAN *et al.* 1989; BLOWS 1993). The two populations reside at, or near, two different adaptive peaks for the trait under investigation and hybrid breakdown indicates that an adaptive valley may lay between them. Therefore, hybrid breakdown provides a way of monitoring the evolution of adaptive peaks in the laboratory. For instance, little is known concerning the depth of fitness valleys between populations. The fitness of a F_2 hybrid does not represent the lowest point in the valley because population fitness would be expected to decrease in subsequent generations until the detrimental effect of recombination between loci is equalled by selection for increased fitness. The final depth of the valley, and how quickly a population will return to a level of fitness equal to or exceeding its original level, will be determined by the number of loci and the rate of recombination between them, the strength of selection and migration rate (the last not being relevant to the present situation) (CROW *et al.* 1990). The level of fitness that was attributable to epistasis (*i.e.*, the depth of the fitness valley between lines) was quite large for development time. Development time decreased on average 12 hr at its lowest point over that found in the parental generations. Furthermore, the bottom of the valley was reached relatively quickly.

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