Effects of Genetic Background on Response to Selection in Experimental Populations of *Arabidopsis thaliana*

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ABSTRACT

The extent to which genetic background can influence allelic fitness is poorly understood, despite having important evolutionary consequences. Using experimental populations of *Arabidopsis thaliana* and map-based population genetic data, we examined a multigeneration response to selection in populations with differentiated genetic backgrounds. Replicated experimental populations of *A. thaliana* with genetic backgrounds derived from ecotypes Landsberg and Niederzenz were subjected to strong viability and fertility selection by growing individuals from each population at high density for three generations in a growth chamber. Patterns of genome-wide selection were evaluated by examining deviations from expected frequencies of mapped molecular markers. Estimates of selection coefficients for individual genomic regions ranged from near 0 to 0.685. Genomic regions demonstrating the strongest response to selection most often were selected similarly in both genetic backgrounds. The selection response of several weakly selected regions, however, appeared to be sensitive to genetic background, but only one region showed evidence of positive selection in one background and negative selection in another. These results are most consistent with models of adaptive evolution in which allelic fitnesses are not strongly influenced by genetic background and only infrequently change in sign due to variation at other loci.

A major goal in evolutionary biology is to understand the genetic basis of adaptive change. Achieving this goal requires knowledge of how natural selection acts on genetic variation and, more specifically, an understanding of the forces that govern the fitness of individual alleles. It is universally accepted among evolutionary biologists that the physical and biotic environments experienced by alleles strongly influence their fitness. The effects of genetic environment (or genetic background) are less clear, however, particularly when variation in genetic background is limited, such as occurs within species. Genetic background is likely to influence the selection values of alleles if fitness depends strongly on gene interactions or epistasis (where fitness of an allele depends on interactions with alleles at other loci; Mayr 1959, 1984; Wright 1964; Wade 1992). Models of adaptive evolution emphasizing gene interactions often view constellations of genes (not individual genes) as the true targets of selection and subsequently view genetic background as an important determinant of allelic fitness.

The relative importance of gene interactions in adaptive evolution has been controversial for some time (Williams 1966; Hedrick *et al.* 1978; Wade 1992; Coyne *et al.* 1997; Fenster *et al.* 1997; Wade and Goodnight 1998; Whitlock and Phillips 2000). This controversy arose largely from the early works of Fisher (1930) and Wright (1931) and their contrasting models of adaptation. At present, the most widely accepted view of evolution (provided by Fisher) holds that most adaptive changes result from selection on the effects of individual alleles (Coyne *et al.* 1997). Fisher's model of mass selection has been more tractable mathematically and perhaps more widely accepted for this reason. This view was challenged by Wright who envisioned gene interactions as crucial in forming an adaptive landscape upon which populations of organisms were thought to exist. In Wright's model, the genetic background experienced by an allele is of critical importance (Wright 1964) because the selection value of the same allele could differ substantially from one genetic background to the next. These underlying genetics were critical to Wright's shifting balance theory of evolution.

Unfortunately, empirical data addressing the importance of gene interactions in adaptive evolution are sparse at best. This is primarily due to the difficulty of estimating the frequency and strength of gene interactions affecting fitness across an entire genome. Moreover, even if substantial interactions affecting important life history traits are detected, as has been reported in some quantitative trait loci (QTL) studies (Doebley *et al.* 1995; Lark *et al.* 1995; Li *et al.* 1997; Shook and Johnson 1999; Leips and Mackay 2000), they are unlikely to change the outcome of adaptive evolution unless the interaction effects of genes exceed their main effects. This is a difficult requirement that has rarely

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been documented empirically (Kim and Rieseberg 2001; but see Long *et al.* 1995). Of course, controversy over the role of gene interactions in adaptive evolution should not minimize their well-documented contributions to other evolutionary phenomena such as the evolution of hybrid incompatibilities among species (Stephens 1949; Rick 1963; Wu and Palopoli 1994; Rieseberg *et al.* 1996), hybrid breakdown and/or divergence among geographically isolated populations (Burton 1990a,b; Armbruster *et al.* 1997; Edmands 1999; Fenster and Galloway 2000), and possibly the maintenance of linkage disequilibrium in natural and artificial populations (Clegg *et al.* 1972; Allard 1975, 1988). Also, reports have suggested that gene interactions may contribute to increased additive genetic variances in populations experiencing bottlenecks (Bryant *et al.* 1986; Goodnight 1988; Bryant and Meffert 1995; Cheverud and Routman 1996) and possibly to increased rates of adaptation in subdivided populations of Tribolium (Wade and Goodnight 1991). As far as we know, however, the measured response of individual genetic factors to parallel selection in different intraspecific genetic backgrounds has not been assessed, particularly on a genome-wide scale. Yet this would provide a means for testing the hypothesis that interactions arising from intraspecific variation in genetic background can strongly influence allelic fitness.

In this report, we use map-based population genetic data to characterize a multigeneration response to selection of experimental populations of *Arabidopsis thaliana.* Replicated experimental populations with genetic backgrounds derived from differentiated ecotypes Landsberg and Niederzenz were subjected to viability and fertility selection over multiple generations by growing individuals from within populations at high density. Selection in these populations was "natural" in that genotypes best able to compete for resources under high-density growing conditions were most successful. Following the generations of selection, the replicated populations were assayed for selection-induced allele frequency changes across the entirety of the genome by determining changes in frequency of mapped molecular markers. We address how genetic background influences the response to selection of individual genetic factors by characterizing how the same genomic regions respond to selection when in the Landsberg and Niederzenz genetic backgrounds.

A. thaliana ecotypes Landsberg and Niederzenz differ phenotypically for a number of life history and fitness-related traits and are distinguishable at multiple diagnostic molecular markers. These ecotypes represent populations that are isolated geographically (they were initially collected in Poland and Germany, respectively) and differentiated genetically. In spite of this divergence, crosses produce fertile progeny, and experimental populations can be constructed in which different chromosomal regions from one ecotype have been transferred into the genetic background of the other via backcrossing. These ecotypes thus provide a good experimental system for addressing how the effects and corresponding selection values of the same genomic regions are influenced when in divergent genetic backgrounds.

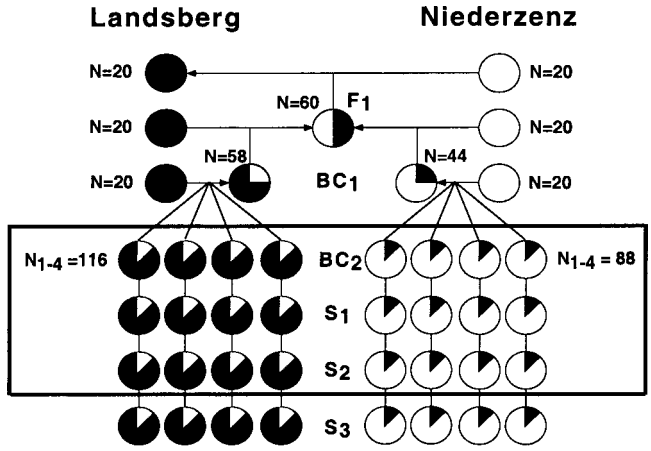

Figure 1.—The construction and propagation of *A. thaliana* experimental populations with Landsberg and Niederzenz genetic backgrounds. Pie diagrams represent populations and illustrate the expected proportions of the Landsberg and Niederzenz genomes on the basis of Mendelian inheritance and in the absence of selection and drift. Arrows indicate direction of pollen movement in artificial crosses. BC_2 populations were initiated with either 116 or 88 seeds. Generations within the rectangular box (BC_2, S_1, and S_2) were subjected to strong viability and fertility selection (see text). Sixty individuals were randomly selected from each S_3 population and genotyped for 31 diagnostic molecular markers to assess genome-wide selection.

MATERIALS AND METHODS

Experimental populations: Seeds of *A. thaliana* ecotypes Landsberg (La-0) and Niederzenz (Nd-1) were obtained from The *Arabidopsis* Biological Resource Center at Ohio State University. To construct the experimental populations, 20 pairwise crosses among Landsberg and Niederzenz individuals were initially conducted and 60 F_1 individuals (three progeny from each initial cross) were backcrossed to each parental type for two additional generations (Figure 1). In the initial cross, Landsberg was used as the maternal parent; F_1 and BC_1 individuals were used as maternal parents in backcrosses. Conducting the latter crosses in this way eliminated pollen competition as a source of gametic selection during backcrossing. Eight experimental populations were initiated in the BC_2 generation: four populations each with 116 Landsberg BC_2 seeds and four populations each with 88 Niederzenz BC_2 seeds (Figure 1). These initial population sizes represent two seeds taken from either 58 or 44 successful second backcrosses toward Landsberg and Niederzenz, respectively. Replicate populations were thus derived from the same seed sources. The experimental populations were genetically variable, but had genetic backgrounds derived primarily from the Landsberg or Niederzenz ecotype (*i.e.*, populations with genomic compositions that were on average 87.5% Landsberg/12.5% Niederzenz or 12.5% Landsberg/87.5% Niederzenz).

The replicated experimental populations (Figure 1) were subjected to three generations of strong viability and fertility selection by growing individuals from within populations at high density in 25 × 25 × 6-cm plastic trays. The Landsberg and Niederzenz ecotypes differ phenotypically for multiple life history and fitness-related traits including flowering time, dry biomass, fruit production, and longevity; the experimental populations were expected to be segregating for this variation during the generations of selection. Throughout these generations (BC_2 through S_2), populations were allowed to self-fertilize. At the end of each generation, seeds were bulk harvested separately from each population and several thousand were used to initiate the next generation in a new tray of equal size. Throughout the experiment, generations were discrete and population gene pools were kept isolated. Seeds from all generations were imbibed and cold treated at 4° for 3 days to break dormancy and promote uniform germination. All generations were grown under 24 hr light in a 25° growth chamber.

Measuring selection response: To infer patterns of genome-wide selection and determine whether genetic background influenced such patterns, a sample of 60 individuals was randomly selected from each experimental population following the last generation of imposed selection. These 480 individuals (60 plants × 4 replicate populations × 2 genetic backgrounds) were genotyped for 31 diagnostic mapped simple sequence repeat (SSR; Bell and Ecker 1994) and cleaved amplified polymorphic sequence (CAPS; Konieczny and Ausubel 1993) genetic markers distributed across all five Arabidopsis chromosomes. These markers cover ~70% of the Arabidopsis genome and intermarker distances average ~16 cM (Figure 2). Prior to selection, expected frequencies of ecotype-specific alleles were 0.875 and 0.125. These initial allele frequencies should be maintained over successive generations in the absence of selection and genetic drift. By examining deviations from these expected frequencies, it was possible to make comparisons both among replicate populations with the same genetic background and between sets of populations with different genetic backgrounds—the latter comparison addressing whether the genetic backgrounds of these Arabidopsis ecotypes influenced the response to selection of ecotype-specific genetic factors. Although segregation distortion in the BC_2 and later generations cannot decisively be ruled out as a source of allele frequency change, it seems unlikely given that 294 F_2 individuals derived from these same lines failed to show any significant segregation distortion at the same set of molecular markers (data not shown).

DNA was extracted from leaf tissue using a scaled version of a CTAB (hexadecyltrimethylammonium bromide) procedure (Doyle and Doyle 1987). PCR amplifications for SSR markers were performed in 15-μl volumes consisting of 1 μl template DNA (~10 ng/μl), 2.4 pmol of each primer, 1 unit of *Taq* polymerase, and a final concentration of 30 mm tricine, 50 mm KCl, 2 mm $MgCl_2$, 100 μm of each dNTP, and 5% acetamide. Reactions were performed on an MJ Research (Watertown, MA) PTC-100 thermal cycler with initial denaturing at 93° for 1 min, followed by 40 cycles of 93° for 1 min, 55° for 1 min, 72° for 1 min, and a final extension of 72° for 9 min. SSR amplification products were separated on either 4% agarose or 5% polyacrylamide, depending on marker allele size differences. For markers scored on polyacrylamide, PCR amplifications were conducted with dCTP labeled with the fluorophore TAMRA (Perkin Elmer, Norwalk, CT) at a final concentration of 0.8 μm and polymorphisms were visualized with a Hitachi FMBIOII fluorescent imager. PCR chemistry of CAPS markers was identical to that of SSR markers, but the temperature cycles followed that of Konieczny and Ausubel (1993). CAPS locus/enzyme combinations are available from the authors upon request. CAPS digest products were separated on 2% agarose, stained with ethidium bromide, and photographed on a UV lightbox.

Frequency deviations arising from genetic drift: Allele frequency deviations may arise from both selection and genetic drift. To determine the magnitude of allele frequency deviations in the experimental populations that could be attributable to genetic drift alone, 95% confidence limits were derived for theoretical predictions of drift variance in populations with both genetic backgrounds, taking account of the multigeneration design of this experiment. Empirical results were then compared to these confidence limits to identify deviations clearly due to selection. After t generations, the expected variance in allele frequencies among replicate populations due to genetic drift is

$$V_{qt} = p_0 q_0 \left[1 - \left(1 - \frac{1}{2N_e}\right)^t\right] \quad (1)$$

(Falconer and Mackay 1996), where p_0 and q_0 are initial allele frequencies (0.875 and 0.125), and N_e is the multigeneration effective population size. The expected variance for the mean of four populations in generation t, $V_{\bar{X}qt}$, is $V_{qt}/4$. A 95% confidence envelope for deviations attributable to genetic drift can thus be expressed as $0.875 \pm (1.96 \times \sqrt{V_{qt}/4})$ and $0.125 \pm (1.96 \times \sqrt{V_{qt}/4})$ for experimental populations with expected allele frequencies of 0.875 and 0.125, respectively.

Over multiple generations, effective population size can be approximated as

$$\frac{1}{N_e} = \frac{1}{t}\left[\frac{1}{N_1} + \frac{1}{N_2} + \frac{1}{N_3} + \ldots + \frac{1}{N_t}\right] \quad (2)$$

(Falconer and Mackay 1996), where t is the number of generations and N_1, N_2, N_3, . . . N_t are population sizes in successive generations. Population sizes were known for three of five generations in which sampling of gametes could have occurred (*i.e.*, BC_1, BC_2, and S_3). For generations in which population sizes were not determined exactly but were known to be large (*i.e.*, S_1 and S_2), estimates of 500 individuals were used and results based on these estimates are shown in our results. To determine the sensitivity to lower estimates for generations S_1 and S_2, estimates of 100 individuals were also used. The estimates of 500 and 500 and 100 and 100 for the S_1 and S_2 generations, respectively, had only minor effects on the placement of 95% confidence limits for deviations attributable to genetic drift.

Estimating selection coefficients: The size of selection coefficient necessary to produce allele frequency changes of a given magnitude can be determined from the equation

$$q_1 = \frac{(q_0^2 + p_0 q_0 F)(1 - s) + (1/2)[(2p_0 q_0 - 2p_0 q_0 F)(1 - hs)]}{(p_0^2 + p_0 q_0 F) + (2p_0 q_0 - 2p_0 q_0 F)(1 - hs) + (q_0^2 + p_0 q_0 F)(1 - s)}, \quad (3)$$

where q_1 is the frequency of an allele in generation $t + 1$, q_0 and p_0 are allele frequencies in generation t (initially equal to 0.125 and 0.875, respectively), F is the coefficient of inbreeding, which, under self-fertilization, changes each generation according to the recursion equation $\frac{1}{2}(1 + F_{t-1})$, and s and h are the selection coefficient and dominance deviation, respectively. Both s and h are bounded by 0 and 1. Equation 3 reduces to

$$q_1 = \frac{q_0^2(1 - s) - p_0 q_0[Fs - 1 + hs(1 - F)]}{1 - 2p_0 q_0 hs + 2p_0 q_0 Fhs - sq_0^2 - p_0 q_0 Fs} \quad (4)$$

and iterations of this reduced equation were performed for three generations over the range 0–0.75 for s (in 0.05 incre-

ments) and for five different values of h (0, 0.25, 0.5, 0.75, and 1).

RESULTS

Effects of genetic background on selection response: Genome-wide patterns of allele frequency change in the experimental populations are illustrated in Figure 2, A–E. A strong deviation from expected allele frequency (strong evidence for selection) is defined as average marker frequencies (associated error bars) that are outside of (nonoverlapping with) the 95% confidence envelope for deviations potentially arising from drift (see Figure 2). Deviations that are suggestive of selection (weaker evidence for selection) are defined as average frequencies (associated error bars) that are outside of (partially overlapping with) the 95% drift confidence envelope.

Genomic regions exhibiting the strongest deviations from expected frequency did so in a similar manner both among replicate populations with the same genetic background and between sets of populations with different genetic backgrounds. For example, Landsberg alleles on the upper region of chromosome 5 (Figure 2E) deviated positively from expected frequency in both genetic backgrounds and Landsberg alleles at the lower region of chromosome 5 deviated negatively. These results suggest that genetic factors on this chromosome had similar effects in all experimental populations and responded to selection in a similar fashion in the two genetic backgrounds. Patterns of background-independent selection were also observed on chromosomes 2 and 4 (Figure 2, B and D). On chromosome 2 (Figure 2B), background-independent selection was observed over regions spanning markers *nga1126*, *m429*, *Bio2b*, and *AthUbique*, with the strongest deviation at marker *nga1126*. Landsberg alleles in this region were at lower-than-expected frequency (negatively selected) in both genetic backgrounds. On chromosome 4 (Figure 2D) Landsberg alleles were at greater-than-expected frequency in both genetic backgrounds, with selection appearing strongest in the interval flanked by markers *AthDET1* and *g3883*. Overall, genomic regions on chromosomes 2, 4, and 5 exhibited the strongest, most consistent responses to selection and presumably harbor genetic factors underlying fitness-related traits.

In contrast, only a single genomic region exhibited a strong selection response that varied across genetic backgrounds. Landsberg alleles at the bottom of chromosome 1 near marker *nga692* deviated positively, on average, in the Niederzenz background but did not deviate from expected frequency in the Landsberg background. Multiple additional regions demonstrated weaker evidence for background-dependent selection on the basis of the criteria described above. For example, Landsberg alleles spanning markers *GapB*, *nga128*, and *AthGENEA* on chromosome 1 (Figure 2A) deviated negatively from expected frequency in the Landsberg genetic background but did not deviate from expected frequency in the Niederzenz genetic background. Similar trends were observed near the middle (marker *AthGAPAB*) and bottom (marker *nga6*) of chromosome 3 (Figure 2C). The region surrounding marker *AthGAPAB* (chromosome 3) is especially noteworthy because it represents the only genomic region where patterns of allele frequency change reversed in the two genetic backgrounds (where Landsberg alleles appeared to be positively selected in one genetic background and negatively selected in another). Again, however, although these deviations are of moderate size, the associated error bars overlap with the 95% confidence envelope for drift.

Selection coefficients: To explore the magnitude of selection coefficients required to observe the genome-wide allele frequency changes in this study, iterations of Equation 4 (see MATERIALS AND METHODS) were conducted for different magnitudes of selection coefficient (s) and dominance (h). Results of those iterations are presented in Figure 3. Two sets of iterations were performed. In both sets, allele frequencies were set initially to 0.125 and 0.875 and iterated for three generations, in accordance with the experimental design of this study. Figure 3A depicts, for different values of selection coefficient (s), expected allele frequencies after three generations for positive selection on an allele at initial frequency of 0.125 and corresponding negative selection on the alternate allele at initial frequency of 0.875. Figure 3B depicts the same information but for negative selection on an allele at initial frequency of 0.125 and corresponding positive selection on the alternate allele at initial frequency of 0.875.

Allele frequency changes are not strongly affected by varying degrees of dominance (h). $h = 0.5$ represents strict additivity (no dominance) and is indicated by solid lines in Figure 3, A and B. $h = 0.25$ and $h = 0.75$ represent equal (and opposite) dominance deviations and $h = 0$ and $h = 1$ represent complete dominance of alternative alleles. Even under complete dominance of alternative alleles ($h = 0$ and $h = 1$), expected allele frequencies after three generations were similar (Figure 3, A and B). The relatively small effects of dominance are not unexpected given that self-fertilizing populations lose one-half of their heterozygosity each generation.

Results from these iterations can be used to determine how large a selection coefficient would be necessary to exceed the 95% confidence envelope for deviations potentially attributable to genetic drift and also to estimate selection coefficients in different genomic regions. The magnitude of selection coefficient necessary to exceed the 95% confidence envelope depends on both the initial allele frequency and whether selection is positive or negative. In the Landsberg genetic background and assuming additive gene action ($h = 0.5$), an allele with initial frequency of 0.875 requires a selection coef-

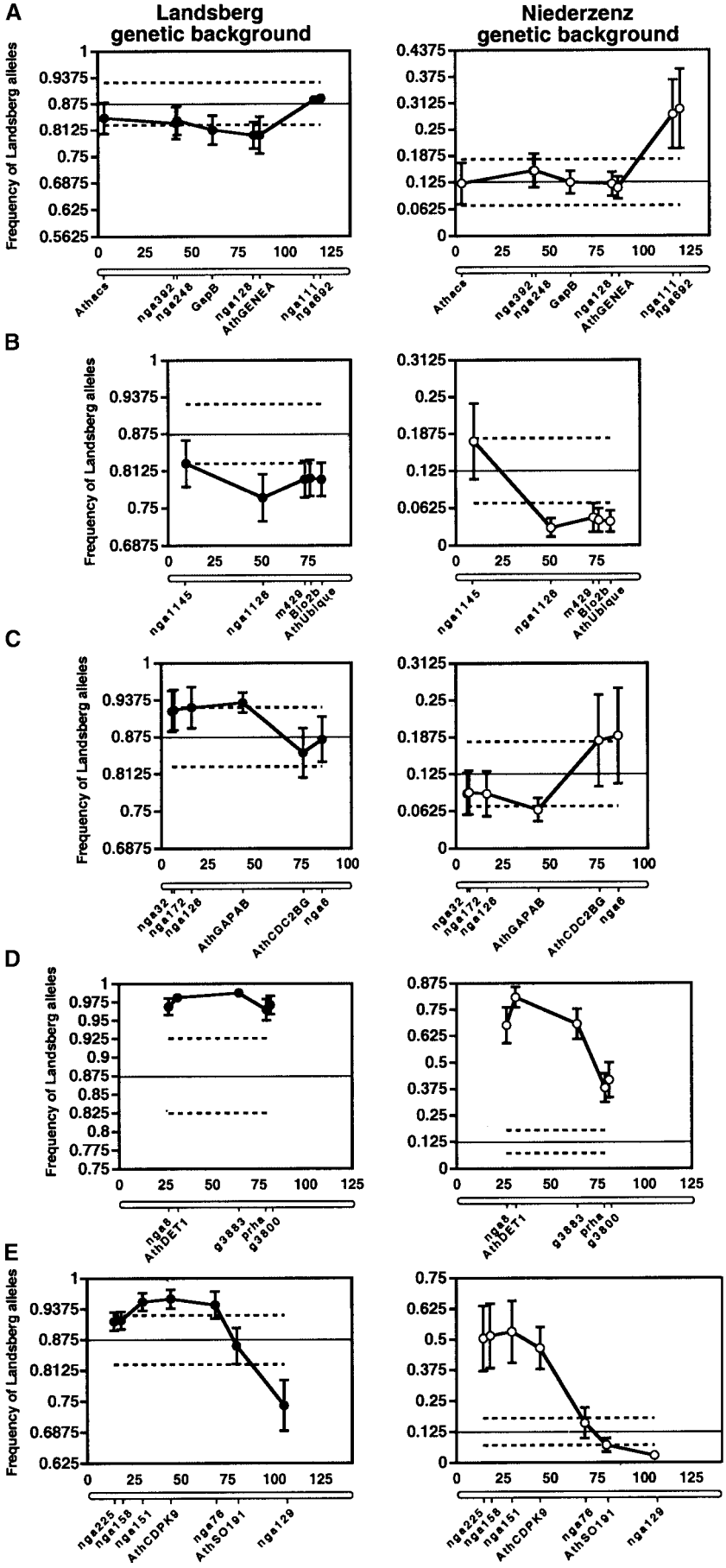

FIGURE 2.—Frequency of Landsberg marker alleles after three generations of viability and fertility selection in experimental *A. thaliana* populations with Landsberg (left) and Niederzenz (right) genetic backgrounds. Symbols connected by lines represent mean allele frequencies (with standard errors) of four populations ($N = 60$ for each population). Where error bars are not indicated, they are less than the height of the symbol. Solid lines at 0.875 and 0.125 indicate the expected frequency of Landsberg alleles in the two genetic backgrounds. Horizontal dashed lines represent a 95% confidence envelope on the basis of theoretical predictions of drift variance. Numbers along the *x*-axis represent distances in centimorgans. Marker positions are based on mapping in recombinant inbred lines derived from a Landsberg *erecta* × Columbia cross (http://nasc.nott.ac.uk/new_ri_map.html). A–E represent chromosomes 1–5, respectively. Note that for chromosomes 4 and 5 (D and E), the allele frequency scale differs between the two backgrounds.

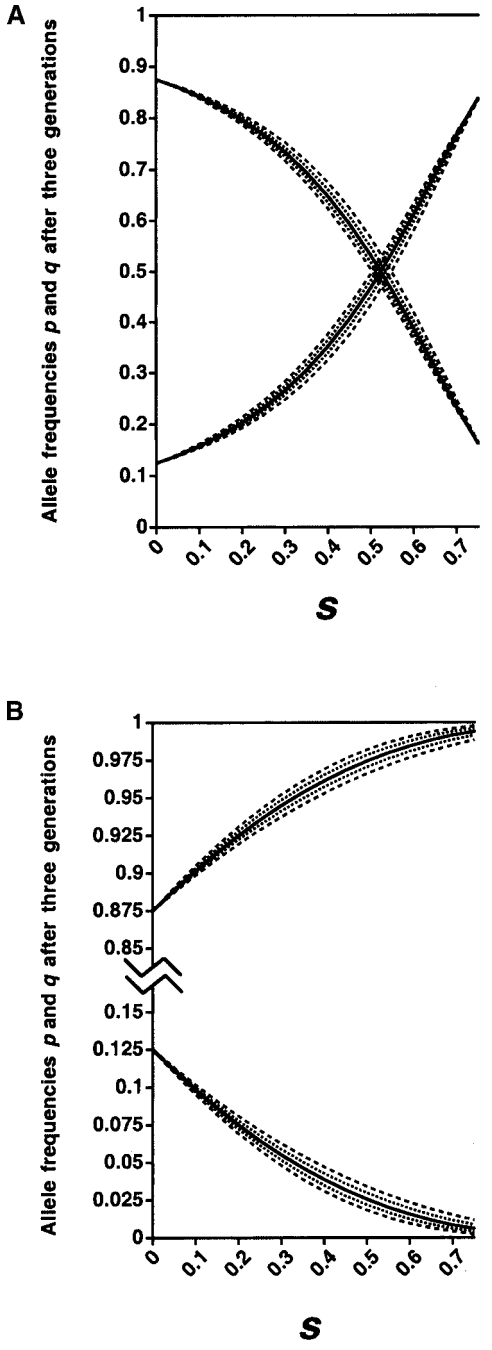

FIGURE 3.—Expected allele frequencies after three generations for various combinations of selection coefficient (s) and dominance (h), assuming self-fertilization and initial allele frequencies of $q_0 = 0.125$ and $p_0 = 0.875$. (—) $h = 0.5$; (···) $h = 0.25$, $h = 0.75$; (- - -) $h = 1$, $h = 0$. (A) Positive selection on q, negative selection on p. (B) Negative selection on q, positive selection on p.

ficient of $\sim s = 0.201$ to exceed the upper 95% confidence limit (CL) and $\sim s = 0.142$ to exceed the lower 95% CL. Likewise, in the Niederzenz genetic background with $h = 0.5$, an allele with initial frequency of 0.125 requires s of $\sim$0.152 to exceed the upper 95% CL and s of $\sim$0.222 to exceed the lower 95% CL. This dependence on initial frequency and direction of selection is attributable to the fact that the effectiveness of selection (the ability of selection to modify allele frequencies) is greater at more intermediate frequencies (FALCONER and MACKAY 1996). In other words, selection is more effective at reducing than increasing allele frequencies that are initially high and more effective at increasing than decreasing allele frequencies that are initially low. Regardless of this difference, however, selection coefficients must indeed be relatively large to exceed the 95% confidence limits in this study.

Selection coefficients also can be estimated for different genomic regions by determining the magnitude of selection coefficient required to produce the observed allele frequency changes. Estimated selection coefficients are given in Table 1 for eight genomic regions. As our estimation procedure necessarily requires, higher selection coefficients are associated with more extreme allele frequency deviations. Although the strength of selection varied from region to region, estimated selection coefficients generally were larger in populations with the Niederzenz genetic background than in populations with the Landsberg genetic background. Selection coefficients were also larger in regions of the genome where selection was similar in the two genetic backgrounds.

Associations among physically linked markers: Selection in different regions of the same chromosome can be influenced by physical linkage, especially for selection on tightly linked loci in repulsion. Correlations of allelic state among physically linked markers were compared to their intermarker map distances (Figure 4). The degree of correlation between pairs of linked markers decreased with increasing intermarker distance. For most chromosomes, the degree of marker correlation was reduced to $< r = 0.5$ for pairs of markers spaced >20 cM apart and $< r = 0.25$ for pairs of markers spaced >40 cM apart.

Heterozygosity: The SSR and CAPS markers used in this study are codominant and thus levels of heterozygosity (and evidence for the presence of overdominance) could be assessed across the entire genome. Heterozygosity was at or near expected levels in all regions (data not shown), providing little evidence for overdominant gene action being involved in the response to selection of the experimental populations.

DISCUSSION

Effects of genetic background on response to selection: The simple yet novel approach of monitoring genome-wide selection in different genetic backgrounds has provided a detailed picture of microevolutionary dynamics in these experimental populations. Evidence both for and against genetic background effects was observed, but it was not observed equally. The response to selection in different genetic backgrounds was similar

TABLE 1

Estimated selection coefficients for eight genomic regions

Genomic region		Selection coefficient (s)[a]	
Chromosome	Marker or marker interval	Landsberg genetic background	Niederzenz genetic background
1	*AthGENEA*	0.194 (0.096–0.269)	0.045 (0–0.147)
1	*nga692*	0.046 (0.004–0.088)	0.340 (0.201–0.438)
2	*nga1126*	0.253 (0.180–0.309)	0.471 (0.359–0.618)
3	*AthGAPAB*	0.239 (0.161–0.329)	0.249 (0.160–0.359)
3	*nga6*	0.013 (0–0.123)	0.171 (0–0.307)
4	*AthDET1–g3883*	0.598 (0.552–0.663)	0.685 (0.647–0.725)
5	*nga151*	0.341 (0.245–0.463)	0.547 (0.449–0.628)
5	*nga129*	0.291 (0.208–0.355)	0.471 (0.353–0.644)

[a] Selection coefficients are estimated from Figure 3, A and B, and are based on the mean frequency deviations of markers or the average of deviations for markers flanking an interval (*i.e.*, *AthDET1–g3883*). Parentheses indicate the range of selection coefficients associated with standard errors for marker frequencies in the experimental populations (see Figure 2) or the average of the standard errors for markers flanking an interval (*i.e.*, *AthDET1–g3883*).

in genomic regions where selection was strongest (*i.e.*, regions showing the largest deviations from expected frequency). The response of one strongly selected region did appear to be influenced by genetic background and trends were evident in multiple additional regions, although selection in these regions was observably weaker. Most importantly, in only one genomic region was there any evidence of a reversal in the sign of selection value across the two genetic backgrounds. Overall, these findings are more consistent with models of adaptive evolution in which selection values of most alleles are not strongly influenced by the genetic background they experience and therefore rarely change in sign.

In instances where genetic background may have influenced the response to selection (*e.g.*, regions on chromosomes 1 and 3), two possible explanations exist. First, these observations could be attributable to different interactions of ecotype-specific alleles in native and foreign genetic backgrounds, as has been hypothesized in models of adaptive evolution invoking strong epistatic fitness (Wright 1931, 1964; Mayr 1959, 1984; Wade 1992). Further study of these regions is certainly warranted to identify the associated phenotypes and to characterize the types of interactions that potentially can give rise to negative epistatic interactions and genetic incompatibilities. It is interesting to note that in two of these regions (bottoms of chromosomes 1 and 3 in the Niederzenz genetic background), variance in allele frequencies was quite high, indicating that smaller-scale genetic background effects may also be evident among replicate populations sharing the same background. The second possible explanation for these observations could be the presence of stabilizing selection on a quantitative trait for which alleles both increasing and decreasing the trait value are segregating (Whitlock *et al.* 1995). Under stabilizing selection the intermediate phenotype is most fit. An allele decreasing the trait value (minus allele) should be negatively selected in a minus allele background where the phenotypic effect of an allele substitution is away from the intermediate optimum. In contrast, a minus allele should be positively selected in a plus allele background where the phenotypic effect of an allele substitution is toward the intermediate optimum. In these circumstances there will indeed be an interaction between allele and genetic background in terms of fitness, although the phenotypic effect of an allele substitution will not change in sign.

Sensitivity of the genome-wide approach: The map-based approach utilized in this study is powerful in that it allows for the analysis of all genes simultaneously and enables quantification of the proportion of the genome that may be sensitive to genetic background effects and the localization of those regions. Such a global analysis, however, necessarily sacrifices local resolution. Chromosomal regions under selection in this experiment were of relatively large size due to the limited number of generations of recombination and the fixation of chromosomal blocks due to selection and selfing. Fragments between 20 and 40 cM were regularly observed at high frequency. Although correlation in allele state between physically linked markers declined with increasing map distance (Figure 4) and thus loosely linked factors could dissociate, conclusions certainly cannot be drawn regarding whether multiple loci were targeted by selection in particular chromosomal regions and if so, whether interactions exist among such loci. Indeed, interactions among tightly linked loci (Jones *et al.* 1977) as well as within a single locus (Stam and Laurie 1996) have been documented and shown to have substantial effects on trait variation. However, given that many more possible interactions exist between a given locus and the rest of the genome than between a given locus and those tightly

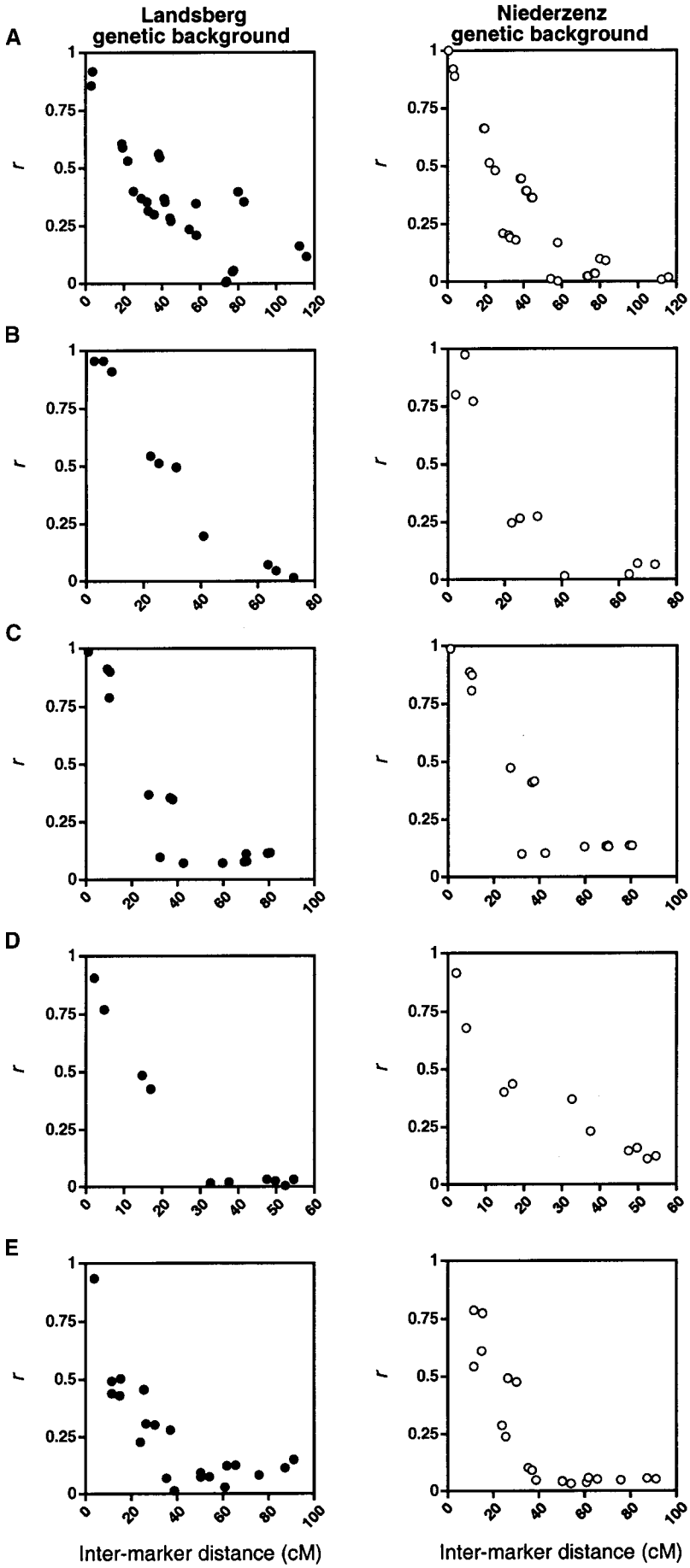

FIGURE 4.—Relationship between the degree of correlation (r) and intermarker distance for pairs of physically linked markers on all chromosomes in the Landsberg (left) and Niederzenz (right) genetic backgrounds. A–E represent chromosomes 1–5, respectively. The correlation coefficient r was calculated as $D/\sqrt{p_A \times q_A \times p_B \times q_B}$ (WEIR 1996), where D is the coefficient of linkage disequilibrium and p_A, q_A, p_B, and q_B represent allele frequencies of markers for which r is estimated. Values of r were obtained using the software package GDA (LEWIS and ZAYKIN 2001), and absolute values, $|r|$, were used for the construction of panels.

linked to it, more potential interactions could be assessed using the methodology of the current study. Furthermore, if interactions arising from different genetic backgrounds are indeed an important determinant of allelic fitness, having multiple linked loci in a foreign genetic background (the case in this study) should magnify any potential genetic background effects because increasing the number of foreign genes in a given background increases the number of possible interactions.

Gene interactions, genetic background, and divergence: Strong genetic background effects have been observed at the interspecific level (Stephens 1949; Rick 1963; Wu and Palopoli 1994; Rieseberg *et al.* 1996), where chromosomal fragments introgressed into the genetic background of another species often have large negative effects on fitness while having neutral or positive effects in a conspecific background. Such interactions can clearly contribute to the maintenance of genetic isolation among species and provide evidence for the "coadaptation," or integration, of species' genomes. However, it is unclear how often such interactions occur within species and whether these interactions frequently influence how selection acts on genetic variation.

Intraspecific crossing studies (King 1955; Enfield 1977; Burton 1990a,b; Armbruster *et al.* 1997; Edmands 1999; Fenster and Galloway 2000) have demonstrated that hybrids derived from geographically isolated populations (or experimental populations selected in parallel) often exhibit either reduced fitness or significant departures from additive and additive/dominance genetic models (but see Cohan 1984; Cohan *et al.* 1989). While many of these empirical studies have discussed their findings in terms of "coadapted gene complexes," they have been unable to demonstrate how natural selection would act on such gene combinations and/or whether the same genetic factors would be differentially selected in different genetic backgrounds. Evidence has also been sought from QTL mapping studies, which have demonstrated that complex interactions among QTL (and in some cases, among chromosomal regions with no observed main effects) can underlie evolutionarily relevant quantitative phenotypes (Doebley *et al.* 1995; Lark *et al.* 1995; Li *et al.* 1997). QTL effects have also been observed to differ across genetic backgrounds (Tanksley and Hewitt 1988; Leips and Mackay 2000). Again, however, experiments to test the dynamics of selection on these interactions have not been conducted. For this study, we deliberately chose compatible but genetically divergent ecotypes to use as raw material in the construction of the experimental populations. The genetic variation within these experimental populations was substantially greater than the published reports of variation found within natural populations of this species (Abbott and Gomes 1989). In spite of this divergence, the most strongly selected factors were selected similarly in both backgrounds, suggesting that at least in these Arabidopsis ecotypes, genetic background may not be a critical determinant influencing how individual genetic factors respond to selection. Of course, it will be necessary to determine whether these conclusions hold for different levels of intraspecific genetic variation (including testing other genetic backgrounds) and for other species and breeding systems. Estimates of species-wide sequence diversity in coding regions among Arabidopsis ecotypes may be only moderate in comparison with other species (data sets are not yet available for large-scale comparisons). If so, species with higher levels of intraspecific (or intrapopulational) genetic diversity will need to be investigated to determine the generality of the results reported here.

A combination of phenotypic analyses and QTL mapping may reveal additional insights into the microevolutionary dynamics of these experimental Arabidopsis populations. Because samples of seeds were taken from generations preceding and following the generations of selection, it should be possible to identify phenotypes that were targeted by selection and to map QTL for those traits. QTL positions can then be compared to genomic regions for which allele frequency changes have been characterized. It should thus be possible to measure selection on specific QTL in the different Arabidopsis genetic backgrounds.

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