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EXPERIMENTAL DEMONSTRATION OF A CAUSAL RELATIONSHIP BETWEEN HETEROGENEITY OF SELECTION AND GENETIC DIFFERENTIATION IN QUANTITATIVE TRAITS

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Abstract.—Comparisons of estimates of genetic differentiation at molecular markers (F_{ST}) and at quantitative traits (Q_{ST}) are a means of inferring the level and heterogeneity of selection in natural populations. However, such comparisons are questionable because they require that the influence of drift and selection on Q_{ST} be detectable over possible background influences of environmental or nonadditive genetic effects on Q_{ST}-values. Here we test this using an experimental evolution approach in metapopulations of *Arabidopsis thaliana* experiencing different levels of drift and selection heterogeneity. We estimated the intensity and heterogeneity of selection on morphological and phenological traits via selection differentials. We demonstrate that Q_{ST}-values increased with increasing selection heterogeneity when genetic drift was limited. The effect of selection on Q_{ST} was thus detectable despite significant genotype-by-environment interactions that most probably biased the estimates of genetic differentiation. Although they cannot be used as a direct validation of the conclusions of prior studies, our results strongly support both the relevance of Q_{ST} as an estimator of genetic differentiation and the role of local selection in shaping the genetic differentiation of natural populations.

Key words.—*Arabidopsis thaliana*, diversifying selection, local adaptation, metapopulation, population structure, uniform selection.

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Local adaptation, arising from spatial or temporal heterogeneity of selection, is a key phenomenon in several important fields of evolutionary biology. It is thought to be one of the mechanisms promoting speciation, both allopatric (Schluter 2001) and sympatric (Via 2001); a central mechanism maintaining adaptive variation (e.g., Levene 1953); and is a major concern in conservation biology for population restoration (e.g., Hufford and Mazer 2003). Local adaptation can be detected from the study of genetic differentiation at potentially selected traits. This differentiation develops as a result of diversifying selection but also through migration and drift (Wright 1951; Spitze 1993). Using the genetic differentiation at neutral molecular markers as a null hypothesis (same drift and migration, no selection), several recent studies exploited comparisons of the differentiation at markers and at quantitative traits to investigate the role of natural selection in shaping the genetic differentiation of natural populations (for reviews, see Merilä and Crnokrak 2001; McKay and Latta 2002). These studies reveal that the differentiation at polygenic traits is, in most cases, significantly larger than that at neutral markers, suggesting a predominant role of local selection in natural populations and widespread local adaptations. These conclusions are in accordance with other estimations of natural selection generally revealing widespread, though moderate, directional local selection (e.g., Kingsolver et al. 2001).

However, such conclusions on the prominent role of local selection on the genetic differentiation of natural populations depend on a number of assumptions underlying the measurement of quantitative genetic differentiation (Merilä and Crnokrak 2001). The latter is classically measured by the Q_{ST} (Spitze 1993), defined as: $Q_{ST} = (1 + F_{IS})V_{BP}/[(1 + F_{IS})V_{BP} + 2V_{WP}]$, where F_{IS} is Wright's (1969) fixation index and V_{BP} and V_{WP} are the between-population and within-population additive genetic variances, respectively. This differentiation index represents the actual genetic differentiation at loci underlying a quantitative trait only if this trait has a purely additive genetic basis with linkage equilibrium among loci (Wright 1951; Lynch et al. 1999). Thus, $Q_{ST} = F_{ST}$ is a theoretical prediction for a neutral trait with additive genetic basis and linkage equilibrium between its underlying loci. Selection heterogeneity modifies the values of Q_{ST} versus F_{ST} via a relaxation of the linkage equilibrium hypothesis. Diversifying selection, for example, tends to create positive associations among loci across populations, which results in an increased between-population genetic variance and larger Q_{ST}-values (Latta 1998). In contrast, under uniform selection, the within-population component of associations among loci is dominant, and small Q_{ST}-values are expected (Le Corre and Kremer 2003).

However, a number of alternative factors, altering linkage equilibrium or the estimation of the additive genetic component of the phenotype, may also lead to a deviation of Q_{ST} from the neutral expectation and therefore to erroneous conclusions regarding the role of selection. First, common environment effects, when measures are conducted in natural

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populations, and maternal effects, in transplant experiments, are responsible for nongenetic resemblance of individuals within populations or family respectively and result in biased genetic differentiation measures. Second, genetic-by-environment (G × E) interactions are likely to interfere with quantitative genetics measures, even in the context of common-garden experiments, either because of microenvironmental variations or because the genetic diversity measured in a seemingly favorable environment might not reflect that in natural populations (Hoffman and Merilä 1999). Such G × E interactions may affect Q_{ST}-values in either way. Third, dominance and epistasis effects have also been demonstrated to affect Q_{ST} (Whitlock 1999; López-Fanjul et al. 2003). Finally, a highly selfing mating system, generating strong within-population linkage disequilibria among loci, can reinforce the effects of the factors listed above. These possible sources of bias, if strong enough, may overcome the effects of local selection and be partly responsible for the conclusion of ubiquitous local adaptation *in natura.* Although theoretically expected, the influence of selection heterogeneity on Q_{ST}-values has never been experimentally demonstrated. Merilä and Crnokrak (2001) and Hendry (2002) therefore recommended additional studies to confirm the predominant role of selection heterogeneity in controlling the level of genetic differentiation at quantitative traits.

Exploring the relationship between Q_{ST}-values and selection heterogeneity would help elucidate the actual influence of local selection versus other mechanisms on the quantitative genetic differentiation measured in natural populations (Merilä and Crnokrak 2001). In particular, checking if the expected positive correlation between Q_{ST} and selection heterogeneity can be masked by additional mechanisms affecting the measure of genetic differentiation is of major importance. To our knowledge, such relationships have never been considered in natural or in experimental populations. Here, we present an experimental evolution approach to test whether Q_{ST} is actually a good indicator of genetic differentiation or if it is mainly driven by environment or nonadditive genetic effects. We followed the evolution of genetic differentiation at nine polygenic traits in 12 initially unstructured metapopulations of *Arabidopsis thaliana,* under different controlled conditions of drift and heterogeneity of selection. The experimental conditions were likely to generate biased measures of genetic differentiation of traits (G × E interactions, possible nonadditive genetic effects, highly selfing mating system), and we monitored the relationship between Q_{ST} and selection heterogeneity in presence of such misleading factors.

Materials and Methods

Experimental Setup: Evolution of the Metapopulations

Twelve metapopulations of *A. thaliana* (each consisting of 20 populations) were grown in the greenhouse for eight generations. The metapopulations differed with respect to population size and heterogeneity of selection (Fig. 1). Six metapopulations were submitted to uniform directional selection for a short life cycle and six metapopulations were under diversifying selection for the duration of life cycle. Diversifying selection was applied in a metapopulation as follows: 10 populations of 20 (numbered 1 to 10) were under selection for a short life cycle, and no artificial selection was imposed on the remaining 10 populations. Populations 1–10 underwent the same selection in the following generations. Within each selection regime, population size within a metapopulation was 10, 25, or 100 plants per population, resulting in total metapopulation sizes of 200, 500, and 2000 plants. Each population size and selection treatment was represented by two replicate metapopulations. Migration between local populations within a metapopulation occurred only through seeds and followed an island model with rate 2%. All populations within a metapopulation could exchange seeds: migration patterns did not depend on the selection for precocity in the diversifying selection regime.

The first generation of the experiment was sampled at random in a pool of F_2 seeds originating from 10 crosses involving 15 plants: 14 individuals from natural populations of *A. thaliana* sampled in the United Kingdom and France (Lavigne et al. 2001), plus one male-sterile mutant (nw77, *pistillata*) used to increase the naturally low outcrossing rate of the species (Snape and Lawrence 1971). We refer to these 15 plants as ''parents'' of the metapopulations. The initial populations were therefore identical on average, except for sampling variability. This variability may have been large in small populations and could be responsible for an appreciable initial level of genetic differentiation.

Each generation, seeds were sown following a regular grid, watered with a solution containing 0.15% fungicide (Dericlor, Ciba Geigy, Basel, Switzerland), and left for one week in the dark at 4°C to break dormancy. Density was constant across population sizes using different pot sizes: 26.4 cm^2 (10 plants), 86.25 cm^2 (25 plants), and 350 cm^2 (100 plants), one population per pot. After germination, the plants were grown in a climate-controlled compartment of a greenhouse under a 16/8 h light/dark photoperiod, 15°C night and 20°C day, and were watered twice a week. The newly sown populations consisted of 88% seeds harvested from hermaphrodites within the population; 10% seeds harvested from male-sterile individuals within the population, which imposed an outcrossing rate of exactly 10% (Porcher et al. 2004); and 2% migrant seeds coming from another randomly chosen population within the metapopulation. Selection for precocity was applied by stopping any watering when the first fruits became mature in either of the two metapopulations within a given selection and population size treatment. In metapopulations under diversifying selection, the subset of populations (numbered 1 to 10) under artificial selection for precocity was the same throughout evolution.

Quantitative Genetics Experiment

We could not measure quantitative traits by directly using plants from the evolving metapopulations, because most measurements were destructive and because estimating the genetic variability of quantitative traits requires individuals of known kinship and replications to control for environmental variation. We therefore used stored seeds from two generations (2 and 8) to analyze the evolution of nine quantitative traits in six populations per metapopulation (Fig. 2). The six populations were randomly chosen with the condition that

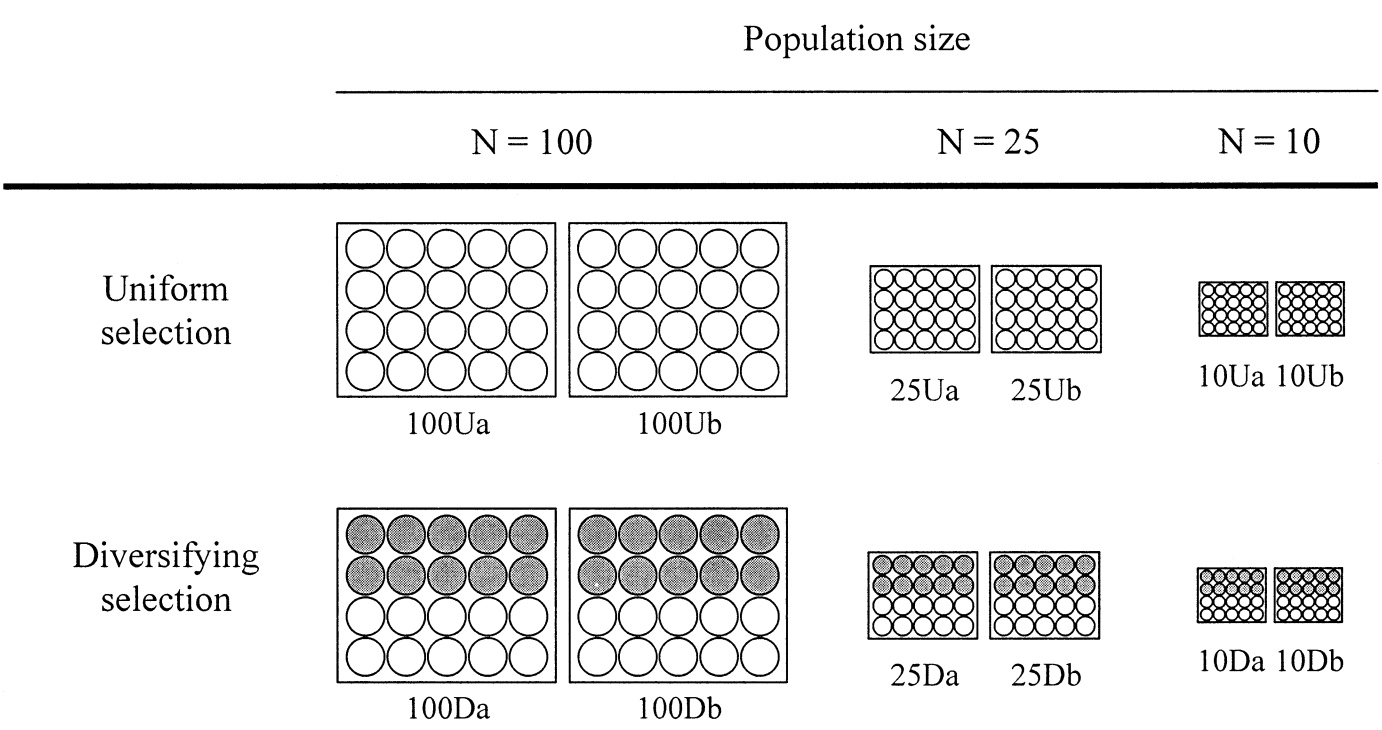

FIG. 1. The 12 experimental metapopulations. A metapopulation (box) consists of 20 populations (circles). Migration follows an island model: the spatial distribution of population does not affect the patterns of gene flow. Metapopulations differ with respect to population size (100, 25, and 10 plants per population) and selection regime: under uniform selection (U), all populations within a metapopulation are selected for a short life cycle (white circles); under diversifying selection (D), 10 populations are selected for a short life cycle (white circles) and 10 populations are not submitted to artificial selection (gray circles). Two replicate metapopulations (a and b) are available for each combination of population size and selection regime.

three populations under selection (i.e., among populations 1–10) and three populations with no selection (among populations 11–20) were sampled from metapopulations under diversifying selection. The same populations were analyzed at generation 2 and 8. Five plants in each population, plus one plant from each of the 15 parental lines were selfed (Fig. 2). Several seeds of each of the 735 resulting families ([2 generations $\times$ 12 metapopulations $\times$ 6 populations $\times$ 5 plants] + 15 parental lines) were sown in separate pots, watered with a solution containing 0.15% fungicide (Dericlor, Ciba Geigy), and left for one week in the dark at 4°C to break dormancy.

When plants reached the four-leaf stage (four leaves, including cotyledons, emerged), four seedlings per metapopulation family and 24 seedlings per parental line were transplanted in 24 large pots (45 $\times$ 30 cm^2), with density conditions similar to those experienced by plants in the metapopulations and with the design explained below. Each pot contained 120 plants (2 sibs $\times$ 5 families $\times$ 6 populations $\times$ 2 generations) originating from selfing of individuals from a single metapopulation. In addition, one plant from each of the 15 parental lines was used as a reference for comparisons between selection treatments (Fig. 2). For each of the 12 metapopulations, two replicate pots were sown. Within a pot, plants from the same generation were grown together to avoid competition between generations, but within a generation plants were randomly distributed (Fig. 2). To prevent border effects, a border of plants coming from the same metapopulation and same generation was sown in each pot. Pots were randomly distributed in the growth chamber and moved around twice a week. The growing conditions were the same as in the metapopulations. Plants of families sampled from a population under directional selection for precocity were harvested when first fruits were ripe within a given metapopulation and a given generation. Plants of families sampled from populations experiencing no artificial selection for precocity were harvested at the end of their life cycle. At harvest, plants were stored on herbarium sheets. Note that this harvesting procedure implies that the plants used for the quantitative genetics experiment experienced the same selection treatment as the populations they originated from, that is, measurement of quantitative traits was not conducted under a common-garden design. Plants from the parental lines were also submitted to two different selection treatments: parental lines grown with plants from metapopulations under uniform selection were harvested together with these plants (selection for precocity); parental lines grown with plants from metapopulations under diversifying selection were harvested at the end of their life cycle (no artificial selection). Because one sibling of each parental line was grown in each pot, a total of 24 siblings were available for each line. Among them,

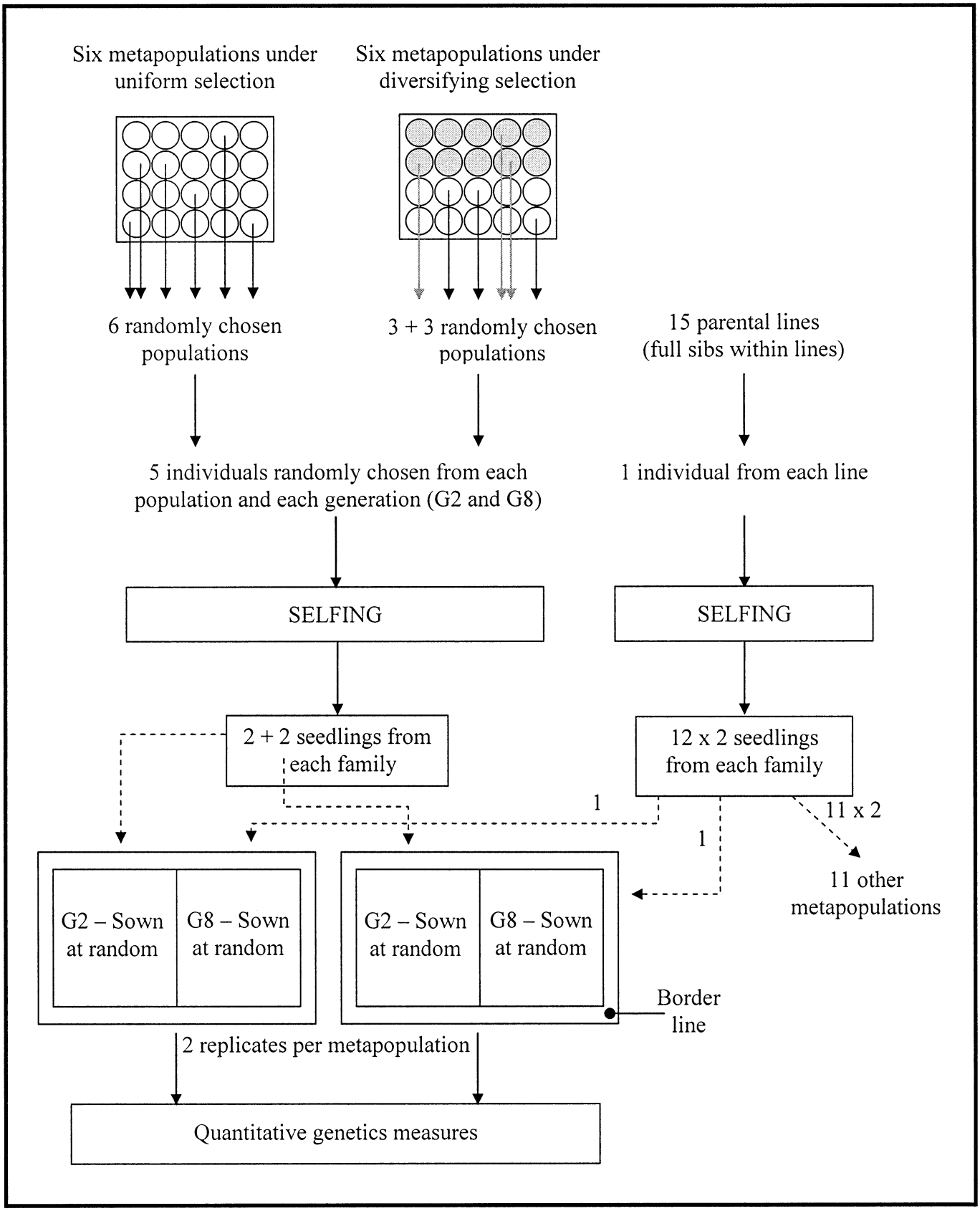

FIG. 2. Experimental design of the quantitative genetics experiment. The sampling procedure used to obtain the families is illustrated, as well as the spatial display within the pots in which the measured individuals were grown. See text for a complete description.

12 were submitted to selection for precocity, 12 were not under such selection.

Two phenological traits and one morphological trait were measured on each individual during the growing period: duration of the rosette stage (DRS, number of days between sowing and bolting), time to flowering (FT, number of days between bolting and flowering), and number of rosette leaves at bolting (NRL). Additional morphological traits were scored on dried plants: plant height (PH), height of the first fruit (HFF), height of the first branch (HFB), number of primary branches (NPB), total number of flowering branches (NFB), and total number of fruits (NF).

Data Analysis

Selection differentials

We estimated the strength of directional selection on each trait at generation 2 using regression methods (Lande and Arnold 1983) for each metapopulation under uniform selection and for each selection treatment, that is, selection for precocity and no selection, within the metapopulations under diversifying selection. The total number of fruits was used as an index of plant fitness, because selfing is predominant in this species, germination rates were close to one and no negative relationship between fruit number and seed number per fruit is generally observed in *A. thaliana* (e.g., Andalo et al. 2001). Following Lande and Arnold (1983), fruit number was transformed to relative fitness by dividing each value by the mean number of fruits produced within the metapopulation or the selection treatment. The values of the eight other traits were divided by their standard deviation within each metapopulation or selection treatment. Standardized selection differentials were calculated as the covariance between relative fitness and each standardized trait. They represent the within-generation phenotypic change in trait mean produced by both direct and indirect selection, notably via correlations between traits. The significance of selection differentials was tested with a Student's *t*-test on six values (3 population sizes $\times$ 2 metapopulations) within each selection regime, because the estimated values did not depend on population size (statistical tests not shown). Each selection differential was estimated on a maximum of 120 (uniform selection) or 60 (diversifying selection) plants; these values lie within the range of usual sample sizes used to estimate the strength of selection (Kingsolver et al. 2001).

Selection heterogeneity was estimated as the standard deviation between two selection differentials. In metapopulations under diversifying selection, the index of selection heterogeneity was computed for each trait as the standard deviation between the selection differential in populations under selection for precocity and the selection differential in populations without such selection. The number of individuals used to estimate selection differentials was small regarding statistical power to detect selection (Kingsolver et al. 2001), due to practical limitations. This is likely to generate sampling variation and thus artificially high selection heterogeneities. We used the metapopulations under uniform selection and resampling methods to estimate such heterogeneity due to random effects. For each metapopulation, we considered all possible combinations of two groups of three populations ($\binom{6}{3}$ = 20 possible combinations). For each combination and each trait, standardized selection differentials were estimated in the two groups of populations. Selection heterogeneity was the standard deviation between these two selection differentials. The mean selection heterogeneity due to random effects was then computed as the average standard deviation over the 20 combinations.

Differentiation of quantitative traits

To estimate variance components, we performed an analysis of variance (ANOVA) on quantitative trait data for each metapopulation separately, using SAS software (SAS Institute 2000), with the model:

$$Y_{ijkl} = \mu + pop_i + fam(pop)_{ij} + rep_k + R_{ijkl}, \quad (1)$$

where Y is the value of a quantitative trait, μ is the mean value of the quantitative trait, *pop* is the population effect ($i = 1 \ldots 6$), *fam* is the family effect ($j = 1 \ldots 5$), *rep* is the replicate effect ($k = 1, 2$), and R is an error variable. Population and family were regarded as random effects. Some residuals were not normally distributed, but the data were left untransformed because no usual transformation improved the normality and ANOVA is known to be robust to deviations from normality (Lindman 1974). Because the families were derived by one generation of selfing from highly homozygous plants (F_{IS} estimated from microsatellite data was always larger than 0.675, data not shown), the between-family genetic variance (V_{BF}) was very close to the within-population total genetic variance (V_{WP}) and the within-family variance (V_R) could be regarded as mainly environmental variance.

We estimated the genetic differentiation at quantitative traits using Wright's (1969) formula: $Q_{ST} = (1 + F_{IS})V_{BP}/[(1 + F_{IS})V_{BP} + 2V_{WP}]$. The within-population variances were estimated from between-family variances as explained above. We used microsatellite data and the Fstat software (Goudet 1995) to estimate F_{IS} (data not shown). Q_{ST}-values were respectively set to zero or one when the estimates of V_{BP} or V_{WP} were negative. Q_{ST}-values were considered significant when the population effect was significant in the above ANOVA.

We tested the population size and selection effects on the Q_{ST}-values within each generation using an ANOVA with the model:

$$Y_{ijkl} = \mu + trait_i + reg_j + size_k + (reg \times size)_{jk} + R_{ijkl}, \quad (2)$$

where $i = 1 \ldots 7$, *reg* is the selection regime ($j = 1, 2$), and *size* is the population size ($k = 1, 2, 3$). The same analysis was also performed within each population size treatment, using a similar model where the *size* effect and its interaction were removed. We did not include all Q_{ST}-values in these analyses because some of the traits under study were highly correlated (Table 1): Q_{ST}-values were therefore dependent variables and the Type I error of the ANOVAs can be biased upward. We removed those traits exhibiting the highest phenotypic correlations (namely number of rosette leaves, correlated with duration of rosette stage, $r = 0.68$; height of the first fruit, correlated with height of the first branch, $r = -0.63$) and conducted the ANOVA on a subset of traits whose

TABLE 1. Phenotypic correlations between quantitative traits at generation 2. Pearson's correlation coefficients were calculated on the whole dataset at generation 2. The sample size was always larger than 1200 individuals. Traits are abbreviated as follows: DRS, duration of rosette stage; NRL, number of rosette leaves; FT, flowering time; PH, plant height; HFF, height of the first fruit; HFB, height of the first branch; NPB, number of primary branches; NFB, number of flowering branches; NF, number of fruits. Traits in bold exhibit high correlations and their Q_{ST}-values are removed from statistical analysis (see text).

	NRL	FT	PH	**HFF**	HFB	NPB	NFB	NF
DRS	**0.68*** **	0.32***	−0.18***	**0.009**	**0.099*** **	0.11***	0.076**	−0.052
NRL		**0.32*** **	**0.12*** **	**0.13*** **	**0.23*** **	**0.21*** **	**0.17*** **	**−0.033**
FT			−0.017	**0.065***	0.10***	0.16***	0.052	−0.041
PH				**0.25*** **	0.29***	0.19***	0.23***	0.15***
HFF					**−0.63*** **	**0.16*** **	**−0.076** **	**0.29*** **
HFB						−0.046	0.32***	−0.15***
NPB							−0.30***	0.017
NFB								0.29***

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

correlations were all smaller than 0.32 (Table 1). Q_{ST}-values for the total number of fruits are included in the above analysis but will not be used in the following for comparisons between Q_{ST} and heterogeneity of selection because selection on this trait cannot be estimated. Note, however, that including all traits or removing one or two additional traits does not modify the significance of the effects presented in the Results section. We did not test the generation effect on Q_{ST} because both generations are also likely correlated.

Potential Sources of Bias for Estimates of Genetic Differentiation

Unlike most studies, the measurements of quantitative traits were conducted in two different environments (defined as the presence or absence of selection on precocity). This was necessary to estimate selection differentials and selection heterogeneity, but is likely to bias the estimates of the genetic differentiation at quantitative traits with environment sensitive expression that may be genotype specific. Morphological traits are in particular more likely influenced by these two environments, because they are built throughout the life cycle of plants and are affected by an early harvesting. Traits linked to plant phenology (i.e., duration of rosette stage, number of rosette leaves and time to flowering), on the contrary, should not be affected because most, if not all, plants flowered before harvesting.

Common-environment effects

While analyzing quantitative traits of metapopulations under diversifying selection, the plants of families sampled from populations evolving in different environments (selection or no selection on precocity) were also measured in these different environments. Therefore, the observed between-population variance may not be influenced by genetic differentiation only, but also by environmental effects. However, Q_{ST}-values under uniform and diversifying selection were not significantly different at generation 2, regardless of population size, and were close to zero (see Results, Tables 2, 3). This suggests that the overestimation of genetic differentiation due to common-environment effects is limited, and we shall not consider these effects in the following.

Genotype-by-environment interactions

We could estimate genotype-by-environment (G × E) interactions in our experiment, using plants from parental lines. These individuals were considered as identical genotypes (clones) within one parental line, because they originated from highly inbred plants ($f \approx 1$ in natural populations of *A. thaliana*; Snape and Lawrence 1971). These clones were grown under both environments. We could therefore test G × E interactions for each trait via an ANOVA with the following model:

$$P_{ijk} = \mu + line_i + env_j + (line \times env)_{ij} + R_{ijk}, \quad (3)$$

where P is the phenotype, *line* the parental line effect ($i = 1 \ldots 15$), and *env* is the environmental effect (selection or no selection on precocity, $j = 1, 2$). *Line* was regarded as a random effect. We also estimated the genetic variance of each trait in each environment, using the parental lines, to quantify the effects of G × E interactions on the estimates of within-population genetic variance.

RESULTS

Strength and Heterogeneity of Selection in the Experimental Metapopulations

In metapopulations under uniform selection, almost all traits were initially subjected to directional phenotypic selection, as indicated by significant selection differentials (Fig. 3); only the height of the first fruit (HFF) appeared to be neutral. In the diversifying selection regime, selection differentials in populations under selection for precocity were similar to those estimated in metapopulations under uniform selection (Student's *t*-tests, no significant differences except for number of flowering branches $t = 2.49$, df = 10, $P = 0.032$). In contrast, within the diversifying selection regime, the strength of selection differed for several traits between populations that did and did not experience artificial selection for precocity. Selection differentials were significantly different between the two environments for height of the first branch (HFB, Student's *t*-tests, $t = 2.43$, df = 10, $P = 0.041$), height of the first fruit (HFF, $t = 3.87$, df = 10, $P = 0.003$), number of rosette leaves (NRL, $t = 3.48$, df = 10, $P = 0.006$) and duration of rosette stage (DRS, $t = 3.65$, df = 10, $P = 0.004$). All these traits were thus under diversifying selection;

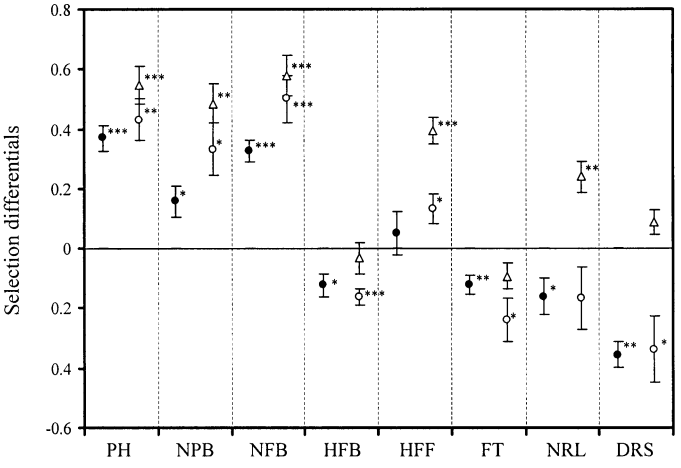

FIG. 3. Estimated selection differentials under uniform and diversifying selection at generation 2. Values are means calculated over the six metapopulations under uniform (filled symbols) or diversifying selection (open symbols). For the latter selection regime, populations submitted to selection for precocity (open circles) were distinguished from populations with no artificial selection (open triangles). Error bars correspond to standard errors calculated over the six metapopulations, and asterisks indicate the significance level for each selection differential: ***$P < 0.001$; **$0.001 < P < 0.01$; *$0.01 < P < 0.05$. PH, plant height; NPB, number of primary branches; NFB, number of flowering branches; HFF, height of the first fruit; HFB, height of the first branch; FT, flowering time; NRL, number of rosette leaves; DRS, duration of rosette stage.

the remaining traits (plant height, PH; number of primary branches, NPB; number of flowering branches, NFB; and flowering time, FT) appear to have been, in fact, under uniform selection even in the diversifying selection regime.

Differentiation of Quantitative Traits

The estimates of genetic differentiation of quantitative traits at generation 2 were close to zero (rare significantly positive values, Table 2) and did not depend on the selection regime (Table 3). However, initial Q_{ST}-values differed among population sizes (Table 3), being significantly higher in metapopulations with 10 plants per population than in metapopulations with larger populations (Duncan test). Q_{ST}-values subsequently generally increased over time for most traits and most metapopulations (Table 2, Fig. 4), except in metapopulations with large population sizes under uniform selection (Fig. 4). At generation 8, the estimated Q_{ST}-values depended on selection regime, with higher values under diversifying selection, especially under large population sizes (Fig. 4). The selection-regime effect on Q_{ST}-values at generation 8 was significant in an ANOVA on all population sizes pooled together (Table 3, selection-regime effect) and within the largest population size treatment only (Table 4, $N = 100$, selection-regime effect). ANOVAs within each population size suggest a decreasing effect of selection regime for small populations (Table 4, Fig. 4, marginally significant effect of population size on Q_{ST}-values at generation 8 in metapopulations with 25 plants per population).

Significant G × E interactions were detected for all traits in the parental lines (ANOVAs on each trait, G × E effect, $P < 0.01$), but their effects of the estimation of variance component were not consistent among traits (i.e., the estimates of within-population genetic variance were either increased or decreased in the absence of selection vs. under selection for precocity). Because the effect of the selection regime on Q_{ST} might be partially attributable to these G × E interactions, we consider in the following Q_{ST}-values at generation 8 and the differences in Q_{ST} between generation 8 and generation 2, to describe the change in genetic differentiation over generations. The use of the difference parameter is likely to remove, at least partially, the G × E interaction effects that must be assumed identical at both generations. When ANOVAs were carried out on this difference, the significance of the selection-regime effect indeed decreased in metapopulations with 25 and 100 plants per population (Table 4), but this effect remained significant for the largest population size ($N = 100$, Table 4).

Genetic Differentiation at Quantitative Traits and Heterogeneity of Selection

To study the relationship between the genetic differentiation of quantitative traits and the heterogeneity of selection expressed in our experimental metapopulations, we performed a linear regression within each population size treatment. This regression related either the Q_{ST} at generation 8 or the change in Q_{ST} between generations 2 and 8 to selection heterogeneity, measured for each trait in each metapopulation. The observed relationship between the change in genetic differentiation and the heterogeneity of selection on a trait depended on population size: the slope of the regression was large and significant under large population size ($y = 0.83x - 0.07$) and decreased under smaller population sizes (Fig. 5). Removing two traits exhibiting high phenotypic correlations (NRL and HFF) from the regression analysis did not modify these results. With six traits only, the equations of the regressions and the associated probabilities were: $y = 0.78x - 0.06$, $P = 0.03$, $N = 100$; $y = 0.40x + 0.21$, $P = 0.39$, $N = 25$; and $y = -0.43x + 0.47$, $P = 0.61$, $N = 10$. When the regression was performed directly between Q_{ST}-values at generation 8 and heterogeneity of selection, on six traits, the same pattern (i.e., lower slope with decreasing population size) was found, although the relationship between estimated differentiation and heterogeneity of selection was weaker for large populations ($y = 0.59x + 0.06$, $P = 0.06$, $N = 100$; $y = 0.79x + 0.27$, $P = 0.18$, $N = 25$; and $y = 0.08x + 0.67$, $P = 0.86$, $N = 10$).

DISCUSSION

The Estimated Level of Differentiation at Quantitative Traits Was Driven by Local Selection

It has been suggested that Q_{ST} may sometimes be a poor indicator of the actual genetic differentiation at quantitative traits because several other factors than heterogeneity of selection and genetic drift influence its estimation (Merilä and Crnokrak 2001; López-Fanjul et al. 2003). Our experimental approach demonstrates that, even in presence of potential factors affecting the measurement of Q_{ST}, the level of selection heterogeneity can indeed determine the level of genetic differentiation at quantitative traits and this differentiation

TABLE 2. Estimated genetic differentiation (Q_{ST}) of quantitative traits. Q_{ST}-values of a trait are given for each population size (100, 25, and 10 plants per population), each selection regime (uniform and diversifying selection for precocity), and each metapopulation (*a* and *b*) at generations 2 (G2) and 8 (G8).

$N = 100$	Uniform selection				Diversifying selection			
	a		*b*		*a*		*b*	
Traits	G2	G8	G2	G8	G2	G8	G2	G8
Plant height	0	0	0	0.03	0.23	0.18	0	0.54
Number of primary branches	0	0.05	0.11	0	0.24	0.25	0	0.57
Number of flowering branches	0.03	0.00	0.08	0.10	0.30*	0.23*	0.13	0.40
Height of the first branch	0	0	0.20	0.26	0.03	0.05	0	0.42
Height of the first fruit	0	0	0.25	0.09	0.08	0.21	0	0.36
Flowering time	0	0.01	0.30*	0.01	0	0	0.16	0.02
Number of rosette leaves	0	0.00	0.33*	0.04	0	0.11	0	0.32
Duration of rosette stage	0	0	0.32*	0.15	0.01	0.09	0	0.23
Number of fruits	0.11	0	0	0.25*	0.12	0.36	0.06	0.29
$N = 25$	Uniform selection				Diversifying selection			
	a		*b*		*a*		*b*	
Traits	G2	G8	G2	G8	G2	G8	G2	G8
Plant height	0	0.20	0.12	0.46*	0.24	0.94***	0	0.32*
Number of primary branches	0.01	0	0.02	0.19	0.22	1	0.38	0.29
Number of flowering branches	0.16	0.43*	0.04	0.43	0.73	1	0.12	0.26
Height of the first branch	0.09	0.08	0.09	0.29	0	0.45	0.10	0.50
Height of the first fruit	0	0.14	0.05	0.27*	0	0.73**	0	0.34*
Flowering time	0	0.04	0	0.55*	0	0.05	0	0.51
Number of rosette leaves	0	0	0.19	0.22*	0.19	0.34*	0	0.33*
Duration of rosette stage	0	0.06	0.14	0.25*	0.08	0.20	0	0.27*
Number of fruits	0.11	0.20	0.16	0.89	1	1	0.01	0.27
$N = 10$	Uniform selection				Diversifying selection			
	a		*b*		*a*		*b*	
Traits	G2	G8	G2	G8	G2	G8	G2	G8
Plant height	0.32*	0.46**	0.17	0.97***	0.24	0.49**	0	0.75***
Number of primary branches	0.23	0.24	0	0.78**	0.33	0.65**	1	0.80**
Number of flowering branches	0.17	0.90***	0	0.89*	0.47	0.68**	0.88	0.53
Height of the first branch	0.25	0.84***	0.04	0.60***	0.00	0.43*	0.12	0.74*
Height of the first fruit	0.25*	0.91***	0.07	0.89***	0.11	0.26	0.08	0.67***
Flowering time	0.56*	0.29	0.27	0.45**	0	1.00	0.51	1.00***
Number of rosette leaves	0.15	0.66***	0.21	0.45**	0	0.48**	0.32	0.79**
Duration of rosette stage	0.21	0.72***	0.26	0.67***	0.04	0.52**	0.29	0.86***
Number of fruits	0.34*	0.73***	0.08	0.71**	0.04	0.40*	0.22	0

*** $P < 0.001$; ** $0.001 < P < 0.01$; and * $P < 0.05$.

TABLE 3. Analysis of variance of Q_{ST}-values within each generation. Effects are abbreviated as follows: *reg*, selection regime; *size*, population size. Significant probabilities ($P < 0.05$) are in bold.

	Effects	df	Mean square	*F*	$P > F$
Generation 2	*trait*	6	0.050	1.22	0.307
	reg	1	0.131	3.23	0.077
	size	2	0.203	4.98	**0.0094**
	reg × *size*	2	0.030	0.75	0.477
	Error	72	0.041		
	Effects	df	Mean square	*F*	$P > F$
Generation 8	*trait*	6	0.040	0.69	0.658
	reg	1	0.340	5.90	**0.0176**
	size	2	1.66	28.78	**<0.0001**
	reg × *size*	2	0.128	2.22	0.116
	Error	72	0.058		

can be accurately described by Q_{ST}. The demonstration is supported by two observations for large population sizes ($N = 100$ plants per population): (1) a significant positive slope between heterogeneity of selection and genetic differentiation, estimated either by a change in Q_{ST} between two generations, or, to a lesser extent, by Q_{ST} at generation 8; and (2) significantly larger Q_{ST}-values under diversifying than under uniform selection at generation 8, compared to a nonsignificant difference at generation 2.

This last significance of the selection-regime effect may be disputable because some of the traits under study were correlated, whereas an ANOVA requires independent observations. However, we removed the traits exhibiting the highest correlations and conducted the ANOVAs on a dataset with minor correlations. In addition, we used a MANOVA on the whole set of Q_{ST}-values to confirm the significance of the selection regime effect (the number of observations was too small to conduct such test on large population sizes only). This analysis revealed a marginally significant effect of selection regime on the genetic differentiation ($P < 0.1$, result

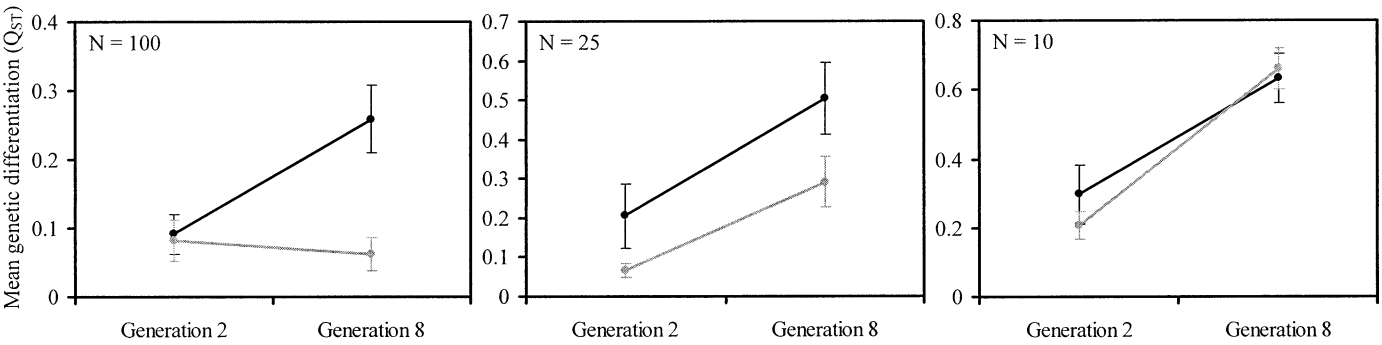

FIG. 4. Changes in the genetic differentiation of quantitative traits in metapopulations under uniform (gray) and diversifying (black) selection. Data correspond to means over seven traits (number of rosette leaves and height of the first fruit were not included) and are plotted for the three population sizes. Errors bars indicate standard errors, calculated over the seven traits. Note that the scale of the y-axes varies across population sizes.

not shown), although small population sizes, in which Q_{ST}-values are not affected by selection heterogeneity, were included. We are therefore confident that selection heterogeneity has a strong influence on Q_{ST}-values under large population sizes.

The existence of a positive relationship between the genetic differentiation, estimated by the Q_{ST}, and the level of selection heterogeneity further suggests that the level of genetic differentiation at quantitative traits not only reveals the presence or absence of diversifying/uniform selection, but also quantifies the degree of selection heterogeneity. The significant effect of selection on Q_{ST}-values could be detected after as few as eight generations of evolution from initially unstructured metapopulations. The experimental metapopulations are probably far from equilibrium by that time; the genetic differentiation may therefore become larger in the following generations of diversifying selection and the observed relationship between heterogeneity of selection and genetic differentiation for large populations is likely to strengthen over time.

Local Selection, Genetic Drift, and the Genetic Differentiation of Populations

Under small population sizes (N = 25 and 10), selection had no detectable effect on the genetic differentiation at quantitative traits. This could be attributable to a classical stronger effect of drift when effective population sizes decrease: the range of population sizes considered in this experimental study generated various situations with respect to the relative influence of selection versus genetic drift. Note that different population sizes also correspond to different numbers of migrants (the migration rate was kept constant): the relative effects of drift versus migration on the genetic differentiation of populations cannot be discriminated here, and these two evolutionary forces are examined jointly. It is generally considered that selection on one locus is more powerful than drift only when the selection differential is much larger than $1/(2N_e)$, where N_e is the effective population size (Wright 1977). This threshold is likely to be modified when polygenic traits are considered, but we can use $1/(2N_e)$ as a reference value to compare population sizes. Under the hypotheses that

TABLE 4. Analysis of variance of the estimated genetic differentiation within population size at generation 8 (G8). Effects are abbreviated as follows: *reg*, selection regime. Significant probabilities ($P < 0.05$) are in bold.

N = 100	ANOVA on Q_{ST}(G8)				ANOVA on Q_{ST}(G8)−Q_{ST}(G2)			
Effects	df	Mean square	F	$P > F$	df	Mean square	F	$P > F$
trait	6	0.022	1.05	0.426	6	0.033	0.97	0.468
reg	1	0.271	13.06	**0.0017**	1	0.247	7.37	**0.013**
Error	20	0.021			20			
N = 25	ANOVA on Q_{ST}(G8)				ANOVA on Q_{ST}(G8)−Q_{ST}(G2)			
Effects	df	Mean square	F	$P > F$	df	Mean square	F	$P > F$
trait	6	0.081	0.92	0.502	6	0.024	0.36	0.834
reg	1	0.318	3.61	0.072	1	0.038	0.58	0.457
Error	20	0.088			20	0.066		
N = 10	ANOVA on Q_{ST}(G8)				ANOVA on Q_{ST}(G8)−Q_{ST}(G2)			
Effects	df	Mean square	F	$P > F$	df	Mean square	F	$P > F$
trait	6	0.033	0.47	0.822	6	0.055	0.37	0.892
reg	1	0.006	0.80	0.777	1	0.096	0.63	0.435
Error	20	0.069			20	0.151		

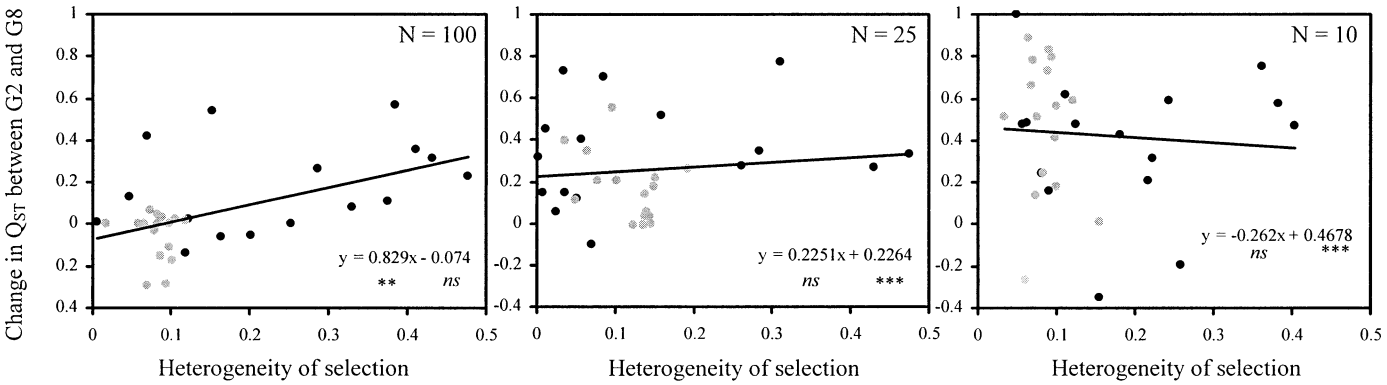

FIG. 5. Observed relationship between genetic differentiation and selection heterogeneity at different population sizes. The change in Q_{ST} between generations 2 and 8 (genetic differentiation) is plotted against the standard deviation between s_1 and s_2 (heterogeneity of selection), where s_1 and s_2 are the standardized selection differentials in two groups of three populations (see text). One value was available per trait and per metapopulation (gray, uniform selection, black, diversifying selection); regressions within each population size were thus calculated on 8 traits × 4 metapopulations = 32 observations. Removing number of rosette leaves and height of the first fruit led to similar results (see text). The significance levels of the slope and of the intercept are indicated below the equations (***$P < 0.001$; **$0.001 < P < 0.01$).

the response to selection in a metapopulation is influenced predominantly by local population size, not total metapopulation size and that effective population sizes depend solely on the breeding system in this experiment, we can roughly estimate effective population sizes as half the census local population sizes (Caballero 1994). Such calculations only generate upper limits for the effective population sizes, since they are likely to be reduced, for example, by variance in reproductive success. The resulting threshold values for selection differentials are 0.1, 0.08, and 0.01 for populations containing 10, 25, and 100 plants, respectively.

Under the smallest population size, selection differentials were close to the threshold values for most traits (mean ± SE absolute value of selection differentials across traits, metapopulations, and selection treatments = 0.25 ± 0.03, 12 of 48 absolute values smaller than 0.1), and population evolution and differentiation were mainly driven by genetic drift, which is consistent with the absence of a significant effect of selection regime on Q_{ST}-values. Under large population sizes ($N = 100$), where all selection differentials exceeded the threshold ($s > 0.01$), the Q_{ST}-values remained constant across generations under uniform selection. This suggests that local selection controlled differentiation. At intermediate population size ($N = 25$), selection and drift were probably both responsible for genetic differentiation (as suggested by an increase in Q_{ST} across generations under both diversifying and uniform selection, but larger Q_{ST}-values under diversifying selection) although selection differentials were far larger than the threshold value (mean ± SE selection differentials = 0.29 ± 0.03, only five values smaller than 0.08).

Genotype-by-Environment Interactions and Possible Other Mechanisms Bias the Estimates of Genetic Differentiation But Do Not Mask Local Adaptation

All the traits we considered were affected by significant G × E interactions, but no clear pattern arose regarding their effect on estimates of within-population variances. It was thus difficult to predict their impact on the estimates of Q_{ST}-values. As theoretically expected, though, G × E interactions markedly affected the estimation of genetic differentiation and partially masked the effect of selection heterogeneity. For example, considering the difference in Q_{ST} between generations 2 and 8, which we assumed removed G × E effects, revealed a stronger relationship between quantitative differentiation and selection heterogeneity than did the Q_{ST}-values at generation 8.

In addition to G × E interactions, several additional factors are likely to have biased the measures of genetic differentiation in this experiment and further clouded the relationship between Q_{ST} and selection heterogeneity. Maternal effects can probably be excluded here because plants were grown under the same conditions for more than eight generations. Nonadditive genetic effects, especially dominance effects due to nonzero levels of heterozygosity within families, are on the contrary likely to act (López-Fanjul et al. 2003), especially for life-history traits (Crnokrak and Roff 1995). Dominance and epistasis effects have been demonstrated for several traits in *A. thaliana* (Kearsey et al. 2003) but their role cannot be determined here. Finally, the highly selfing mating system of *A. thaliana* may also cause an alteration of Q_{ST}-values, via strong within-population linkage among quantitative trait loci and a possible strengthening of the other mechanisms affecting genetic differentiation or its estimation. In contrast, selfing is expected to increase response to selection because of higher parent-offspring correlation and hence be responsible for stronger differentiation between populations under diversifying selection. The role of selfing on Q_{ST}-values has not been theoretically thoroughly examined, especially in interaction with other mechanisms. Le Corre and Kremer (2003) demonstrated that such effect is limited for a trait with a purely additive genetic basis and unlinked quantitative trait loci, but it might be strengthened when environmental or nonadditive genetic effects also act.

Nonetheless, the relationship between selection heterogeneity and genetic differentiation remained detectable via the measurement of Q_{ST}-values, despite evidenced effects of G × E interactions and likely, though not demonstrated, consequences of other mechanisms on estimates of genetic differentiation.

Consequences for the Study of Local Adaptation in Natural Populations

Our results tend to confirm the conclusion drawn from the general observation of large Q_{ST}-values relative to F_{ST}-values in natural populations, that local selection plays a major role in shaping the genetic differentiation at quantitative traits (Merilä and Crnokrak 2001; McKay and Latta 2002). The rationale behind the use of experimental metapopulations as a model of natural populations is that, although these metapopulations were grown under artificial selection, the estimated strength of selection was comparable to the strength of natural selection (mean standardized selection differential = 0.28 in our study vs. mean standardized selection gradient = 0.22 in a review of directional selection in natural population by Kingsolver et al. 2001). Moreover, the effective population sizes in the experimental metapopulations are probably similar, if not smaller, than classical effective population sizes in natural populations (maximum local effective population sizes between five and 50 individuals, compared to estimated effective population sizes in natural plant populations ranging from three to several thousands individuals; Schoen and Brown 1991; Milligan et al. 1994 and references therein). Consequently, the influence of selection on the genetic differentiation in the experimental metapopulations cannot be attributed to an unusually strong selection versus drift. In fact, the Q_{ST}-values observed in the large populations of this experiment were comparable to those in natural populations. The mean Q_{ST} observed in natural populations is 0.38, ranging between 0.06 and 0.96 (McKay and Latta 2002); the mean Q_{ST} in our experimental metapopulations under large population size and diversifying selection was 0.26, ranging between zero and one. Q_{ST}-values were somewhat larger under smaller population sizes (Fig. 4), probably due to a stronger effect of drift. Note, in addition, that the genetic differentiation in the experimental metapopulations is unlikely to have reached equilibrium after eight generations.

As in this experimental study, Q_{ST}-values estimated from natural populations are most probably not influenced by selection and drift only, and additional mechanisms could be responsible for the observed large Q_{ST}-values. The study of genetic differentiation in natural populations of plants is based on measurements of Q_{ST} either directly in natural populations (notably for tree species; Yang et al. 1996) or more frequently in common-garden experiments (e.g., Bonnin et al. 1996; Kuittinen et al. 1997; Steinger et al. 2002; Widen et al. 2002). The estimation of genetic differentiation is therefore likely affected by common environment effects, when measures are conducted *in natura*, and also by G × E interactions, for both types of measures. Second, when the generation time of the study species is long and the parents of the measured individuals cannot be grown in the common environment (e.g., Kremer et al. 1997), maternal effects are likely to act. Finally, most studies focus on a mixture of life-history traits and morphological traits, sometimes life-history traits only (see Merilä and Crnokrak 2001). The genetic basis of life-history traits includes a significant part of nonadditive variance (Crnokrak and Roff 1995), a third mechanism possibly affecting Q_{ST} (López-Fanjul et al. 2003).

Using experimental metapopulations, we have demonstrated that selection heterogeneity was the main mechanism controlling Q_{ST} when genetic drift was limited. These experimental metapopulations experienced selection and drift intensities comparable to those in natural populations. Moreover, as in natural populations, several factors suspected to bias the estimates of genetic differentiation were present (strong G × E interactions, possible nonadditive effects, and high selfing rate). The observation of a significant effect of selection heterogeneity on Q_{ST} in experimental metapopulations is not a direct proof that these possible sources of bias remain weak in comparison to selection in natural populations. However, because this experimental approach mimics the conditions of measurement of the genetic differentiation of natural populations, our results provide strong support for the idea that the large Q_{ST}-values observed in natural populations indeed reflect local adaptation originating from diversifying selection.

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Literature Cited

Andalo, C., I. Goldringer, and B. Godelle. 2001. Inter- and intragenotypic competition under elevated carbon dioxide in *Arabidopsis thaliana*. Ecology 82:157–164.

Bonnin, I., J.-M. Prosperi, and I. Olivieri. 1996. Genetic markers and quantitative genetic variation in *Medicago truncatula* (Leguminosae): a comparative analysis of population structure. Genetics 143:1795–1805.

Caballero, A. 1994. Developments in the prediction of effective population size. Heredity 73:657–679.

Crnokrak, P., and D. A. Roff. 1995. Dominance variance: associations with selection and fitness. Heredity 75:530–540.

Goudet, J. 1995. FSTAT (vers. 1.2): a computer program to calculate *F*-statistics. J. Hered. 86:485–486.

Hendry, A. P. 2002. $Q_{ST} > = \neq < F_{ST}$? Trends Ecol. Evol. 17: 502.

Hoffmann, A. A., and J. Merilä. 1999. Heritable variation and evolution under favourable and unfavourable conditions. Trends Ecol. Evol. 14:96–101.

Hufford, K. M., and S. J. Mazer. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends Ecol. Evol. 18:147–155.

Kearsey, M. J., H. S. Pooni, and N. H. Syed. 2003. Genetics of quantitative traits in *Arabidopsis thaliana*. Heredity 91:456–464.

Kingsolver, J. G., H. E. Hoekstra, J. M. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. Am. Nat. 157:245–261.

Kremer, A., A. Zanetto, and A. Ducousso. 1997. Multilocus and multitrait measures of differentiation for gene markers and phenotypic traits. Genetics 145:1229–1241.

Kuittinen, H., A. Mattila, and O. Savolainen. 1997. Genetic variation at marker loci and in quantitative traits in natural populations of *Arabidopsis thaliana*. Heredity 79:144–152.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Latta, R. G. 1998. Differentiation of allelic frequencies at quantitative trait loci affecting locally adaptive traits. Am. Nat. 151: 283–292.

Lavigne, C., X. Reboud, M. Lefranc, E. Porcher, F. Roux, I. Olivieri, and B. Godelle. 2001. Dynamic management of genetic resources in metapopulations: *Arabidopsis thaliana* as an experimental model. Genet. Sel. Evol. 33:S399–S423.

Le Corre, V., and A. Kremer. 2003. Genetic variability at neutral markers, quantitative trait loci and trait in a subdivided population under selection. Genetics 164:1205–1219.

Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. Am. Nat. 87:331–333.

Lindman, H. R. 1974. Analysis of variance in complex experimental designs. W. H. Freeman & Co., San Francisco.

López-Fanjul, C., A. Fernández, and M. A. Toro. 2003. The effect of neutral nonadditive gene action on the quantitative index of population divergence. Genetics 164:1267–1633.

Lynch, M., M. Pfrender, K. Spitze, N. Lehman, J. Hicks, D. Allen, L. Latta, M. Ottene, F. Bogue, and J. Colbourne. 1999. The quantitative and molecular genetic architecture of a subdivided species. Evolution 53:100–110.

McKay, J. K., and R. G. Latta. 2002. Adaptive population divergence: markers, QTL and traits. Trends. Ecol. Evol 17:285–291.

Merilä, J., and P. Crnokrak. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. J. Evol. Biol. 14: 892–903.

Milligan, B. G., J. Leebens-Mack, and A. E. Strand. 1994. Conservation genetics: beyond the maintenance of marker diversity. Mol. Ecol. 3:423–435.

Porcher, E., P.-H. Gouyon, and C. Lavigne. 2004. Dynamic management of genetic resources: maintenance of outcrossing in experimental metapopulations of a predominantly inbreeding species. Conserv. Genet. 5:259–269.

SAS Institute. 2000. SAS/STAT user's guide. Ver. 8. SAS Institute, Inc., Cary, NC.

Schluter, D. 2001. Ecology and the origin of species. Trends Ecol. Evol. 16:372–380.

Schoen, D. J., and A. H. D. Brown. 1991. Intraspecific variation in population gene diversity and effective population size correlates with the mating system in plants. P. Natl. Acad. Sci. USA 88:4494–4497.

Snape, J. W., and M. J. Lawrence. 1971. The breeding system of *Arabidopsis thaliana*. Heredity 27:299–302.

Spitze, K. 1993. Population structure in *Dahpnia obtusa*: quantitative genetic and allozymic variation. Genetics 135:367–374.

Steinger, T., P. Haldimann, K. A. Leiss, and H. Müller-Schärer. 2002. Does natural selection promote population divergence? A comparative analysis of population structure using amplified fragment length polymorphism markers and quantitative traits. Mol. Ecol. 11:2583–2590.

Via, S. 2001. Sympatric speciation in animals: the ugly duckling grows up. Trends Ecol. Evol. 16:381–390.

Whitlock, M. 1999. Neutral additive genetic variance in a metapopulation. Genet. Res. 74:215–221.

Widen, B., S. Andersson, G.-Y. Rao, and M. Widen. 2002. Population divergence of genetic (co)variance matrices in a subdivided plant species, *Brassica cretica*. J. Evol. Biol. 15:961–970.

Wright, S. 1951. The genetical structure of populations. Ann. Eugenics 15:323–354.

———. 1969. Evolution and the genetics of populations. Vol. 2. The theory of gene frequencies. Univ. of Chicago Press, Chicago.

———. 1977. Evolution and the genetics of populations. Vol. 3. Experimental results and evolutionary deductions. Univ. of Chicago Press, Chicago.

Yang, R. C., F. C. Yeh, and A. D. Yanchuk. 1996. A comparison of isozyme and quantitative genetic variation in *Pinus contorta* ssp. *latifolia* by F_{ST}. Genetics 142:1045–1052.

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