Adaptation and extinction in experimentally fragmented landscapes

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Competition and disturbance are potent ecological forces that shape evolutionary trajectories. These forces typically work in opposition: when disturbance is infrequent, densities are high and competition is intense. In contrast, frequent disturbance creates a low-density environment in which competition is weak and good dispersal essential. We exploited recent advances in genomic research to quantify the response to selection by these powerful ecological forces at the phenotypic and molecular genetic level in experimental landscapes. We grew the annual plant Arabidopsis thaliana in discrete patches embedded in a hostile matrix and varied the number and size of patches and the intensity of disturbance, by creating both static and dynamic landscapes. In static landscapes all patches were undisturbed, whereas in dynamic landscapes all patches were destroyed in each generation, forcing seeds to disperse to new locations. We measured the resulting changes in phenotypic, genetic, and genotypic diversity after five generations of selection. Simulations revealed that the observed loss of genetic diversity dwarved that expected under drift, with dramatic diversity loss, particularly from dynamic landscapes. In line with ecological theory, static landscapes favored good competitors; however, competitive ability was linked to growth rate and not, as expected, to seed mass. In dynamic landscapes, there was strong selection for increased dispersal ability in the form of increased inflorescence height and reduced seed mass. The most competitive genotypes were almost eliminated from highly disturbed landscapes, raising concern over the impact of increased levels of human-induced disturbance in natural landscapes.

Arabidopsis thaliana | competition | disturbance | evolution | genomics

Natural selection should lead to adaptive evolution in response to ecological forces. However, in spatially structured landscapes, competition and disturbance can exert conflicting selection pressures. For example, high disturbance rates lead to reduced densities and reduced competition but favor those traits that confer good dispersal ability. In contrast, low disturbance rates lead to high density and intense competition, favoring traits that confer good competitive ability (1–3). However, although competition and disturbance both lead to exclusion and loss of diversity, coexistence can theoretically occur in multispecies communities at some intermediate disturbance level (4–7).

Although the conditions for coexistence have been a subject of great debate (7), the relative strengths of disturbance and competition as forces shaping the phenotypic and genetic composition of plant communities have rarely been examined in an experimental setting. Here we used communities composed of multiple genotypes of Arabidopsis thaliana (L.) Heynh. to address this question. We considered this to be an appropriate model for an ecological community because natural populations of Arabidopsis thaliana are >95% self-fertilizing (8); hence, like a group of species, cooccurring lines mostly produce seeds of a genotype identical to the parent. In addition, recombinant inbred line (RIL) populations are available for Arabidopsis (9); these populations have no coevolutionary history, so that the success of genotypes can be more easily linked to particular genes and traits. Moreover, changes in the frequency of alleles can be measured using high-throughput genomic methods, localized to specific regions of the chromosomes and related to known quantitative trait loci (QTLs) for the relevant traits (10, 11).

We constructed 24 independent experimental landscapes in a glass house, each consisting of multiple habitat patches embedded in a hostile matrix. Because our primary focus was not coexistence, we imposed two disturbance regimes (static and dynamic) representing the extreme ends of the disturbance gradient: in static landscapes patches were never disturbed, whereas in dynamic landscapes all patches were destroyed every generation and only dispersing seeds survived. To create static landscapes, nondispersing seeds were collected from the surface of existing patches, and all dispersing seeds falling into the matrix were destroyed (Fig. 1A). To create dynamic landscapes we collected dispersing seeds by placing randomly arranged Petri dishes of the same size and number as the original patches within the matrix and destroyed all existing patches at the end of each generation (Fig. 1A). In all landscapes, patches were then randomly relocated, refilled with new soil, and sown with seeds collected from the previous generation. Landscapes consisted of 2, 4, 8, or 16 patches, with the combined patch area held constant at approximately 7% of the total [similar to the percentage of bare earth naturally occurs (12)]. We included a patch number treatment because increasing the number of patches decreases the average dispersal distance and allows a greater heterogeneity to develop among patches, potentially slowing competitive exclusion.

We seeded the landscapes with a selection of RILs derived from the large-seeded Cape Verde Islands (Cvi) and the small-seeded Landsberg erecta (Ler) accessions (13, 14). We chose this population because it exhibits a seed size/number tradeoff (15) and because competition/colonization tradeoffs have often been cast in terms of seed size (16, 17): large-seeded species are suggested to have superior competitive ability and small-seeded...
species superior colonizing abilities (because they produce more seeds). In addition, because inflorescence height will inevitably be linked to dispersal distances, we exploited the presence of the *erecta* mutation in this population, which greatly reduces inflorescence height. Of the 19 lines selected, 10 carried the *erecta* mutation and 9 carried the wild-type *erecta* allele (SI Appendix, Table S1). When grown under our conditions the mean mass per 100 seeds ranged from 2.34 mg to 5.07 mg among lines. Among the selected lines, seed mass was unaffected by the *erecta* mutation ($t = 0.156, df = 17, P = 0.8$), whereas inflorescence height was considerably reduced ($t = 5.94, df = 17, P < 0.0001$); thus, selection can act mostly independently on inflorescence height and seed mass. After seeding the 24 landscapes in generation 1, they were allowed to evolve independently for five generations with no mixing among landscapes.

**Results**

**Population Density.** Seedling and adult densities were recorded in all landscapes in each generation. In static landscapes, mean seedling densities were higher [static: 700.7 (95% CI, 614–787); dynamic: 341.5 (95% CI, 255–428)], as expected if most seeds fall close to the parent plants, and a much smaller fraction of those seedlings survived to adulthood as compared with dynamic landscapes ($F_{1,20} = 76.99, P < 0.0001$). To test whether the reduced survival of seedlings in static landscapes is due to higher seedling densities, we refitted the model with seedling density fitted first as covariate. Seedling density had a highly significant negative effect on survival ($F_{1,208} = 557.8, P < 0.0001$), and fitting this covariate removed the significant difference between static and dynamic landscapes ($F_{1,20} = 1.43, P = 0.25$), supporting the idea that the reduced survival observed in static landscapes is caused by increased competition. Seedlings also survived better in landscapes with larger patch sizes ($F_{1,20} = 73.67, P < 0.0001$), but there was no effect of patch size on seedling densities ($F_{1,20} = 0.53, P = 0.47$; SI Appendix, Table S2).

**Phenotypic Changes.** Measurements of plant height and seed mass in each generation indicated that static and dynamic populations gradually diverged through time (SI Appendix, Fig. S1), although there were no clear or consistent effects of patch size. To remove the confounding effects of density, seeds were sampled from individuals growing in all 24 landscapes in generation 5 and sown in single cells. To control for maternal effects, seeds from these individuals were then collected and resown in single cells alongside four individuals from each of the 19 lines forming the original, ancestral population. After growing in standardized conditions, plants sampled from dynamic landscapes were clearly taller than those from static landscapes, a difference that was due to two separate phenomena (Figs. 1B and 2). First, the percentage of individuals carrying the *erecta* mutation was much lower in dynamic landscapes compared with static ones [static: 44.1% (95% CI, 40.8–47.5%) *erecta*; dynamic: 7.81% (95% CI, 6.04–9.85%) *erecta*]. Second, both mutant and wild-type individuals were taller in dynamic landscapes: individuals carrying the *erecta* mutation were on average 2.5 (95% CI, 0.088–5.9) cm taller, whereas wild-type *ERECTA* individuals were on average 9.0 (95% CI, 7.4–10.6) cm taller (SI Appendix, Table S2).

Contrary to our expectation that competitive environments would favor large seeds, the average seed mass in static landscapes [mass of 100 seeds: 2.65 (95% CI, 2.14–3.16) mg] was not significantly different ($F_{1,20} = 2.98, P = 0.099$) from that in dynamic ones [mass of 100 seeds: 2.39 (95% CI, 2.18–2.61) mg]. However, populations in all landscapes experienced selection for lighter seeds compared with the ancestral population [mass of 100 seeds: 3.32 (95% CI, 3.10–3.55) mg; Fig. 2]. Nevertheless, populations from static landscapes had significantly higher variance in seed mass ($F_{1,22} = 14.5, P < 0.001$), owing to the higher frequency of large-seeded individuals: for example, the percentage of individuals with seed mass greater than the ancestral mean was 22.4% in static landscapes but only 4.1% in dynamic landscapes. It therefore seems that much stronger directional selection occurred in dynamic landscapes, leading to greater loss of phenotypic diversity.

**Genome-Wide Genetic Changes.** To characterize the observed phenotypic changes at the genotypic level we carried out a genome-wide genetic analysis of the 24 populations using rapid array mapping (10). In a RIL population a maximum of two different alleles are present at any given locus (18) because all lines are derived from only two homozygous parental accessions (in our case Ler and Cvi); hence, we can examine changes in the frequency of Ler and Cvi alleles across the genome in populations after selection. First, we used an efficient genotyping approach using ATH1 Affymetrix Genechips to identify genes that harbor two different alleles in the parental accessions (ArrayExpress, accession no. E-MTAB-107)—so-called single feature polymorphisms (SFPs) (19). SFPs could be any genetic mismatch (e.g., deletion, nucleotide changes) that results in significant differences in hybridization intensities between the two parental lines on the microarrays. Comparing the normalized and averaged microarray

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signal ratios between the two parents (Ler and Cvi), we mapped 26,638 SFPs at a false discovery rate of 4.8%, allowing us to unambiguously distinguish the Ler and Cvi alleles at more than 26,000 loci. This provided a very dense physical map with 213 molecular markers per megabase across the Arabidopsis genome. For each of the SFP markers, bulk segregant analysis (10) was carried out using microarrays to calculate allele frequencies for each of the 24 populations after selection relative to the ancestral population. To have roughly equal contributions of genetic material from all individuals within each population, we picked one flower per progeny of each generation-5 plant grown under standardized conditions. Within each of the 24 populations, flowers were pooled for DNA extraction and microarray hybridization. For each population, the normalized data were first scaled according to the differences in mean hybridization intensities of the parents. Scaled signals from the ancestral population (17 RILs plus two parental lines) were subtracted to correct for any bias in allele frequencies in the ancestral population, and the data were LOESS smoothed. If there was no change in allele frequencies, ratios should center on zero (homozygous), whereas if Cvi alleles were preferentially selected, ratios should move toward +0.5 (homozygous Cvi), and if Ler alleles were preferentially selected, ratios should move toward −0.5 (homozygous Ler).

Generally, allele frequencies in populations from both static and dynamic landscapes moved toward Ler (Fig. 3); however, the average shift in dynamic landscapes was significantly greater than in static ones, and populations from static landscapes were significantly more homozygous, again suggesting that directional selection has been less intense (SI Appendix, Fig. S2). We compared our profiles with the positions of previously identified QTLs (14, 20–22), which are found across the Arabidopsis genome and affect many phenotypic traits, including seed mass and plant height (Fig. 3 and SI Appendix, Fig. S3). Six major QTLs (Fig. 3, green rectangles) have been identified in Arabidopsis, which together influence an estimated 77% of all phenotypic traits analyzed to date (20). All but one of these major QTLs map to regions that show significant allele frequency shifts toward Ler in populations from dynamic landscapes but remain homozygous in populations from static landscapes. The major QTL on chromosome 2 maps to the erecta mutation, an important regulator of plant height, and is the only part of the genome that has significantly shifted toward Cvi in dynamic landscapes. The frequency of the erecta mutation in the ancestral population was 52.6%. In populations from dynamic landscapes the genetic estimate of the mean frequency of erecta plants was 15.61% (95% CI, 9.96–21.26%), compared with 46.12% (95% CI, 36.5–55.74%) in populations from static landscapes, in good agreement with the phenotypic data. It therefore seems that the erecta mutation was selectively neutral in static landscapes but experienced strong, negative selection in dynamic ones. Allele frequencies along the entire length of chromosome 4 have shifted toward Ler in both landscape types. Previously mapped QTLs for plant height and seed mass on chromosome 4 are not focused around a major QTL (compare with chromosomes 1, 2, 3, and 5, where they are mostly found to colocalize with major QTLs) but are instead distributed across the entire chromosome (SI Appendix, Fig. S3).

Genotypic Changes. Each RIL has a unique pattern of Ler and Cvi alleles (9), yielding a distinctive chromosomal signature and allowing us to identify successful lines. We genotyped individuals from both 16-patch static (n = 120) and 16-patch dynamic (n = 118) landscapes, which revealed that lines generally bred true, although four recombinant individuals were found. To assess the possible magnitude of drift, we simulated genotypic diversity in generation 5 assuming that, in each generation, genotypes were selected at random according only to their frequency in the previous generation. Population sizes were constrained to the known adult population sizes. We repeated this 5,000 times for each landscape to construct a confidence interval for the expected genotypic diversity under drift alone (SI Appendix, Fig. S4). The simulations reveal that the observed diversity loss in both static and dynamic landscapes far exceeds that expected under drift alone (SI Appendix, Fig. S4).

In dynamic landscapes there was strong selection for genotypes producing a tall inflorescence; for example, 43.1% of all individuals in dynamic landscapes had inflorescence height >350 mm, and such plants in the ancestral population (Fig. 2) belong to only three RILs. In generation 5, two of these three lines (CvL39 and CvL125) made up 90% of all genotyped individuals (Fig. 4). Both lines have very small seeds and consist predominantly of Ler alleles: CvL125 carries Ler alleles at an estimated 90% and CvL39 at 77% of the genome; hence they are genetically identical at 78% of the genome.

In populations from static landscapes, 10 of the original 19 lines were found (Fig. 4), although, unlike in dynamic landscapes, these genotypes vary considerably in both seed mass and inflorescence height. In a separate experiment, we therefore measured size-standardized growth rates on the 17 RILs plus the Ler parent (23). Growth rate was a very good predictor of abundance in static landscapes (F₁,₁₃ = 14.56, P = 0.0019), unlike inflorescence height (F₁,₁₃ = 1.14, P = 0.31), the presence of the erecta mutation (F₁,₁₃ = 0.73, P = 0.41), or seed mass (F₁,₁₃ = 1.71, P = 0.21). Rapid growth is likely to be selected when there is intense scramble competition for resources, as is likely to occur among synchronously germinating annual plants (24, 25). The two most successful lines in static landscapes were the small-seeded
Ler parent and the large-seeded CVL168 (Fig. 4). These two fast-growing genotypes made up 49% of individuals in static landscapes, but they were almost eliminated from dynamic landscapes, presumably because they are both short and hence have poor dispersal ability.

Discussion

After only five generations, populations in static and dynamic landscapes had diverged both phenotypically and genetically and were dominated by different genotypes. This supports the basic assumption of competition/colonization tradeoff models, that competition and disturbance select for fundamentally different traits, and that a single species (or genotype) is unlikely to be both a good competitor and a good colonizer. However, although both competition and disturbance exerted directional selection, leading to exclusion and loss of diversity, disturbance was the more potent force in this regard. In contrast, the effects of patch size were weak and nonsignificant, perhaps because the limited scale of our experiment precluded such important ecological phenomena as edge effects, in which individuals growing near the edge of patches are particularly disadvantaged.

Competition/colonization tradeoff models usually seek to explain the coexistence of species with different traits, which we did not assess here; for example, if large seeds have increased competitive ability (16, 17), then a competition/colonization tradeoff model can potentially support multiple seed size strategies. However, the mean seed mass in static landscapes declined relative to the ancestral population, making this an unlikely explanation (26). Instead, success in static landscapes was strongly correlated with growth rate. In highly disturbed or dynamic landscapes, two very tall, small-seeded genotypes almost exclusively dominated. Inflorescence height was clearly the most important trait for success in dynamic landscapes, because small-seeded genotypes with short inflorescences (those carrying the erecta mutation) were not successful. Such rapid evolution for good dispersal ability probably occurs because a small difference in mean height can greatly increase the chances of a seed dispersing a relatively long distance (27, 28); hence, short genotypes were heavily penalized in dynamic landscapes. It is noteworthy that the sensitivity of highly competitive genotypes to disturbance and their loss from dynamic landscapes is also predicted by competition/colonization tradeoff models (29). Thus, the increasing levels of human-induced disturbance in natural habitats can be expected to have phenotypic and genetic consequences.

Recent theoretical developments in community ecology have suggested alternative models of community diversity (30) that rely on equalizing tradeoffs (31)—to reduce fitness differences among species with different traits—coupled with ecological drift. However, despite small population sizes, selection was still the dominant force in our experiment. This demonstrates that traits closely linked to fitness, such as height, seed size, and growth rate, are unlikely to be selectively neutral, even in the presence of tradeoffs that tend to equalize fitness differences (32). Our results also demonstrate that although certain traits or genes may be close to neutral in one particular environment (e.g., the erecta mutation in static landscapes), it is highly unlikely that this neutrality will be preserved under any major environmental change. Thus, the extreme fragility of neutral models makes them unlikely candidates for the long-term maintenance of trait diversity.

Materials and Methods

Landscape. Landscapes were trays measuring 90 × 64 cm and 7 cm deep, filled with a sand/soil mixture. Landscapes contained 2, 4, 8, or 16 circular patches (cylindrical slices of PVC tubing cut to the same depth as the tray) with diameters of 17.5 cm, 11 cm, 8 cm, or 5.7 cm, respectively, thus keeping the total suitable area roughly constant at approximately 7%. Landscapes were set up in a glass house and subjected to two levels of disturbance (static or dynamic). Patches were located in a stratified random way and relocated in each generation. Each landscape was sown with 16 seeds of each of the 19 lines in generation 1, but in subsequent generations dispersing seeds (dynamic landscapes) and nondispersing seeds (static landscapes) were collected and transferred to the next generation (Fig. 1). Each landscape was

Fig. 3. Allele frequency shifts along chromosomes for dynamic and static landscapes. Features were plotted on the physical map (Mb); the genetic Map (cM) is given as reference. The mean of the three replicate populations within each of the eight treatment combinations is shown. The threshold for significant frequency shifts toward Cvi alleles is +0.17, and the threshold for shifts toward Ler alleles is −0.17 (11, 33). The position of previously identified major QTLs (20) and QTLs for seed weight, seed length, and plant height (13), as well as centromeres, are indicated. Arrows mark the 2-Logarithm of Odds (2-LOD) support interval of QTLs. Left-pointing arrows indicate that the Cvi allele increases the phenotypic value of the trait, right-pointing arrows that the Cvi allele decreases the phenotypic value of the trait. Information on the variance in a QTL affecting a particular phenotypic trait has been published previously (22) and is not included in the figure.
were genotyped after conditions and grew these 30 individuals a second time in the same way. The plants for the genetic analysis described below (54 Biology, University of Zürich). A single mixture used in the main experiment) to exclude the effects of density on the selection. (A) In the ancestral population, 19 lines were sown at equal frequency. Colors reflect the percentage of alleles in each RIL inherited from the Landsberg (Ler) parent (pink = high percentage Ler alleles; green = low percentage Ler alleles). In static (B) and dynamic (C) landscapes, a sample of individuals from the 16-patch landscapes (static: n = 120; dynamic: n = 118) were genotyped after five generations of selection.

**Phenotypic Assessment.** In generation 5, a single siliques was removed from 77 randomly selected plants in each landscape. Seeds from different siliques were not mixed, and we regrew the 77 individuals from each landscape under randomly selected plants in each landscape. Seeds from different siliques were measured. We kept a minimum daytime temperature of 22 °C and a minimum night temperature of 20 °C. Additional lighting came on automatically when daylight fell below 25 klx and hence we ensured a 16-h day in all generations.

**RILs.** The RIL population is described fully elsewhere (13, 14). We selected lines for this experiment by sampling across the entire available seed mass range with a balance of mutant erecta and wild-type ERECTA lines.

**Genotypic Assessment.** Bulk segregant mapping with array genotyping has been described in detail elsewhere (11). Analysis scripts were adapted as follows (available upon request): Because the parental accessions were Ler and Cvi and not Columbia (Col), which is the basis of the targets located on the ATH1 Affymetrix Genechips, two-tailed modified t tests were performed instead of one-tailed. To minimize the possibility that identified SFPs represent duplications between Cvi and Ler accessions, potential SFP duplications were mapped using a two-tailed t test on previously published microarray data for Col/Ler and Col/Cvi. Hybridization signals that were significantly higher in the Ler or Cvi accessions compared with the Col accession indicate duplications. A threshold of 15,011 (Col/Ler) and 15,133 (Col/Cvi) SFP hits was used to identify 1,382 and 754 potential duplications, respectively (the total number of SFP hits for Cvi/Ler was 27,692, which was considered the minimum combined threshold). Of these 2,136 potential duplications, 1,937 were unique and 1,054 SFP markers matched in the Cvi/Ler population and were thus removed from further analysis, leaving a total of 26,638 SFP markers for bulk segregant analysis. Raw hybridization data for mapping duplications are available upon request. Simulation studies have been used to set appropriate thresholds for significant allele frequency shifts for different genetic models and pool sizes (11, 33). We used a threshold of 400 SFPs represent duplications between Cvi and L

**Mapping of Genetic Markers.** Because QTL data were published using genetic markers (cm), and the microarray data were generated using the published Arabidopsis sequence (AGI, 125 Mb), we mapped the genetic markers onto the nucleotide sequence. Two different genomic maps were used for mapping QTLs and genotyping RIL lines. For QTL mapping using Col/Ler RIL lines (21, 22) we used the NASC genetic Map (http://arabidopsis.info/new_ri_map.html) with a total of 1,288 genetic markers. Markers with known primer sequences were mapped to the genome sequence using BLAST analysis. All remaining markers for known nucleotide positions were identified by searching the TAIR database (http://www.arabidopsis.org/index.jsp). In total, 590 genetic markers were mapped to their physical position, which corresponds to a density of 1.212 kb. Physical markers that mapped on or close to the published QTL positions were used for mapping QTLs on the genome sequence. For QTL mapping of Cvi/Ler-derived RILs and Cvi/Ler RIL genotyping we used the published AFLP Cvi/Ler RIL map (9). This map consists of 292 genetic markers, of which 115 were previously mapped to unique nucleotide positions in the genome (34). For the remaining markers not previously mapped, the nucleotide position was estimated by interpolation with the position of the closest marker for which the nucleotide position was known. Using this information, markers used in RIL genotyping as well as QTL markers were mapped to their respective positions in the genome sequence. We noticed a shift of exactly 1 Mb on chromosome 4 in the originally published physical AFLP map (34) compared with the published AGI sequence and corrected marker positions accordingly. All data in the figures were plotted on the genome sequence scale (Mb); the genetic scale (cm) is given as reference only.

**Genotyping Lines.** We sampled seeds from 40 different generation-5 plants in each of the 16-patch landscapes (three static and three dynamic). Seeds were plated out on MSagar, and 120 seedlings per landscape type were grown for 14 d before DNA was extracted (35) (96-well format) for genotyping. To distinguish the 17 RIL lines and the two parental lines, we tested nine polymorphic genomic loci (36) that gave different fragment size on amplification (fragment length polymorphism) in the Ler and the Cvi parental.
lines using PCR. A summary of the markers used, the PCR primers, and the resulting fragment length polymorphism is provided in SI Appendix, Table S3. PCR was first carried out on DNA of the 17 ancestral RIL lines to establish the reference genotype. The PCR conditions were as follows: 94 °C for 120 s (1x); followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s (primer 9 for 90 s), 72 °C for 45 s; and 72 °C for 10 min (1x). The size of PCR products was analyzed on a 2% agarose gel.


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