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Rapid evolution accelerates plant population spread in fragmented experimental landscapes

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Abstract:
Predicting the speed of biological invasions and native species migrations requires understanding the ecological and evolutionary dynamics of spreading populations. Theory predicts that evolution can accelerate species' spread velocity, but how landscape patchiness, an important control over traits under selection, influences this process is unknown. We manipulated the response to selection in populations of a model plant species spreading through replicated experimental landscapes of varying patchiness. After six generations of change, evolving populations spread 11% further than non-evolving populations in continuously favorable landscapes, and 200% further in the most fragmented landscapes. The greater effect of evolution on spread in patchier landscapes was consistent with the evolution of dispersal and competitive ability. Accounting for evolutionary change may be critical when predicting the velocity of range expansions.

One Sentence Summary: Evolution on ecological timescales increases the velocity of experimental plant populations spreading through patchy habitats.

Main text:
In an era of global environmental change, biological invasions and the movement of species ranges with climate change present two of the greatest threats to natural and managed ecosystems (*1, 2*). At the core of each dynamic is the spread of populations across landscapes fragmented by natural and anthropogenic barriers to movement. That habitat fragmentation slows the velocity of spread has long been appreciated (*3, 4*), but its influence on the potential for evolution to increase population expansion is unknown (*5*). Theory shows that natural selection at the low-density front of populations expanding through continuously favorable landscapes coupled with the spatial sorting of offspring favors traits contributing to fecundity and dispersal, both of which accelerate the invasion velocity (*6-10*). Whether this eco-evolutionary process operates similarly in systems fragmented by unsuitable habitat is uncertain because spread in these systems depends on the build-up of high density populations capable of dispersing over gaps (*5, 11*). Any factor that alters selection on an expanding population can influence spread, but whether evolution through selection or genetic drift predictably affects spread velocity on the rapid time scale of ecological dynamics remains an open question. Answering such questions has

important implications for predicting the future spread of biological invasions and climate change migrants.

Empirical progress toward understanding evolution in populations spreading through fragmented landscapes is limited, largely because the process occurs over many generations and at geographic spatial scales. Due to these constraints, nearly all empirical evidence for evolution affecting spread comes from a few retrospective, observational analyses (*12*-*16*). The spread velocity of cane toads, for example, increased 6-fold after introduction to Australia, consistent with evolved changes in dispersal (*14, 17, 18*). Nonetheless, with stochastic events contributing to the ecological and evolutionary trajectories of spreading populations (*5, 19*-*21*), replicated, controlled studies are necessary for understanding the predictability of this eco-evolutionary dynamic (*15*). Given the challenges of replicating invasions in the field and doing so in landscapes of varying fragmentation, model laboratory systems present an excellent opportunity to evaluate how evolution affects the speed at which populations expand through habitats of varying patchiness.

We manipulated evolution in populations of the model plant *Arabidopsis thaliana* spreading through continuous and fragmented landscapes, each consisting of a linear array of rectangular pots (Fig. 1A; *22*). We initiated each replicate invasion in the left-most pot of the array by sowing equal fractions of 14 genotypes (recombinant inbred lines), which varied in spread-relevant traits. Due to nearly complete self-pollination of *A. thaliana* (*23*), the 14 genotypes can be treated as clones (*24*), facilitating our measurements of evolutionary change. In evolving populations, the resulting plants produced seeds, which dispersed across the array (assisted via a simulated rain event), constituting the next generation of the population (Fig. 1B). In non-evolving treatments, germinants emerging in the next generation were replaced with individuals randomly drawn from the initial seed pool, thus maintaining population dynamics while eliminating any change in the frequency or spatial sorting of genotypes. We manipulated habitat patchiness by separating individual pots of suitable habitat by gaps that were 0 (continuous landscapes), 4, 8, or 12 times the mean dispersal distance. This protocol was repeated over six generations of spread, at which point individuals at the leading edge and back of the invasions were genotyped, and traits of all 14 genotypes measured.

We found that after six generations of spread in continuous landscapes, evolving populations spread a modest 11% further than non-evolving populations (Fig. 2A), a difference that was only marginally significant ($t_{13.5} = -2.05$, $P = 0.060$). By contrast, in experimental landscapes with gaps 12 times the mean dispersal distance, evolving populations spread three times as far as their non-evolving counterparts (Fig. 2D; $t_{10.4} = -3.36$, $P = 0.007$), leading to a significant gap size by evolution interaction ($F_{1,72} = 10.77$, $P = 0.002$). The effects of evolutionary change were so strong in patchy landscapes that evolving populations showed no significant reduction in velocity as the size of gaps increased from 4 to 8 to 12 times the mean dispersal distance (generation 6 location of dark green line in Figs. 2B, C and D; $F_{1,25} = 0.014$, $P = 0.908$), even as velocity slowed in the non-evolving populations ($F_{1,28} = 8.52$, $P = 0.007$). Patchiness and evolutionary change also influenced the among-replicate variability in expansion velocity (Fig. 2). The coefficient of variation for spread was four times greater in the patchiest landscapes than in the continuous ones (fig. S1), consistent with a spread process driven by infrequent long-distance dispersal events in fragmented systems. We also found that evolving

populations showed significantly less among-replicate variation in spread than non-evolving populations (fig. S1). Thus, despite the theoretical expectation for greater genetic drift at the leading edge of spreading populations (*25*), invasion speed was more predictable in evolving populations.

One explanation for the greater effects of evolutionary change on spread velocity in patchier landscapes might be faster evolution due to stronger selection in these systems. However, the extent of genotypic change did not differ significantly with gap size (fig. S2, Table S1; Fig. 3 shows the initial and final genotypic compositions), and the extent of trait change increased only marginally with increasing gap size (Fig. 3, fig. S2, Table S1). In fact, trait and genotypic change occurred in populations spreading through all landscape types, irrespective of whether evolution enhanced the spread velocity (significant intercepts in the fitted models of Table S1). These evolutionary changes reflect the combined effects of selection and drift. In the continuously favorable landscapes in particular, we found more among replicate variation in the genotypic composition of leading individuals than expected by chance (fig. S3), consistent with spatial priority effects where genotypes that initially got ahead due to chance dispersal were able to stay ahead (*5, 25*).

Despite similarities in the extent of trait and genotypic change across gap sizes, landscape patchiness affected the direction of evolution. Height and the average distance of the furthest dispersed seed, traits correlated with one another ($r_s = 0.55$, $P = 0.046$), increased with landscape patchiness (backward and rightward shift of the replicates with increasing patchiness in Fig. 3, $P = 0.008$, $P = 0.060$ respectively, Table 1). These trait changes were associated with changes in the genotypic composition of the leading individuals with increasing patchiness (Fig. 3; $F_{1,34} = 2.54$, $P = 0.042$). Given theory showing that greater dispersal increases the invasion velocity (*6-10*), the evolution of greater height and dispersal in patchier systems is consistent with the greater effects of evolution on spread in these landscapes. Nonetheless, whether landscape patchiness selected directly for better dispersal or indirectly via unmeasured traits that are correlated with dispersal remains an open question.

Increased competitive ability likely also contributed to the greater effects of evolutionary change on spread velocity in patchier systems. Although competitive ability evolved to the same extent regardless of gap size (upward shift of replicates in Fig. 3, Table 1; a similar result was found for seed mass), theory (*5, 11*) predicts increasing competitive ability will have a greater effect on spread in fragmented than in continuously favorable landscapes (fig. S4 shows this result applied to our system). In fragmented habitats, individuals often compete at crowded invasion fronts, enabling genotypes that make more offspring at high density (i.e. better competitors) to spread faster (*5, 11*) (fig. S4). Though weaker, this effect also emerges in models of finite populations in continuously favorable landscapes (fig. S4; *26*), consistent with the evolving populations moving modestly further than the non-evolving populations in continuous landscapes (Fig. 2A).

Extrapolating our results to wild populations requires care for several reasons. First, the focal populations were effectively asexual, meaning that trait variation was not continuous and traits were perfectly linked. Nonetheless it is not clear how more continuous variation or less linkage between traits would influence the effect of evolutionary change on spread velocity.

Second, we note that while we manipulated genetic change in this experiment, we cannot rule out the influence of maternal and epigenetic effects on our results. Third, we explored the effects of fragmentation assuming it has no effects on the initial pool of genetic variation. If fragmentation in the non-spreading portion of a species range were to select for reduced dispersal (*16, 27*), then populations that spread from such sources might have less genetic variation in dispersal-related traits, limiting the response to selection. Related to this point, the effects of evolution in our study arose through drift and selection on standing variation; our results do not bear on the rates of evolution resulting from the rise of novel mutations.

In conclusion, our results demonstrate that evolution on ecological timescales can increase the speed of advance in spreading populations. Our results show it can do so markedly in the most patchy landscapes, though further studies are needed to evaluate whether patchiness per se generally selects for traits that increase spread (*24*). Our results for less patchy landscapes show that large evolutionary changes in spreading populations can have little or no consequence for spread velocity. More generally, our results add a more process-focused perspective to past work which has shown either accelerating invasion fronts consistent with evolution (*13-15, 17*) or trait differences between individuals at the front and back of spreading populations (*18, 28, 29*). We conclude that accounting for evolutionary change on ecological time scales may be critical when predicting the rate at which biological invasions and climate change migrants reach new locations.

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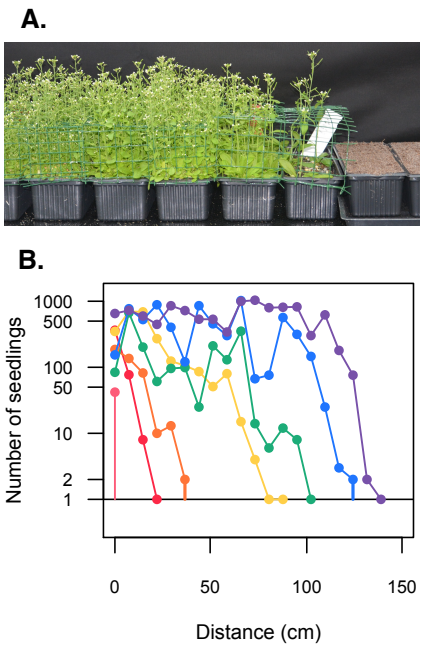

Fig. 1. Spread of *Arabidopsis thaliana* in experimental greenhouse arrays
A. Leading edge of an invasion of a continuous landscape. B. Spread in a continuous landscape for one replicate in the evolving treatment. Each colored line represents one generation (pink = founding population, red = 1st generation of spread to purple = 6th generation of spread), and points show abundance in the individual pots that make up the arrays.

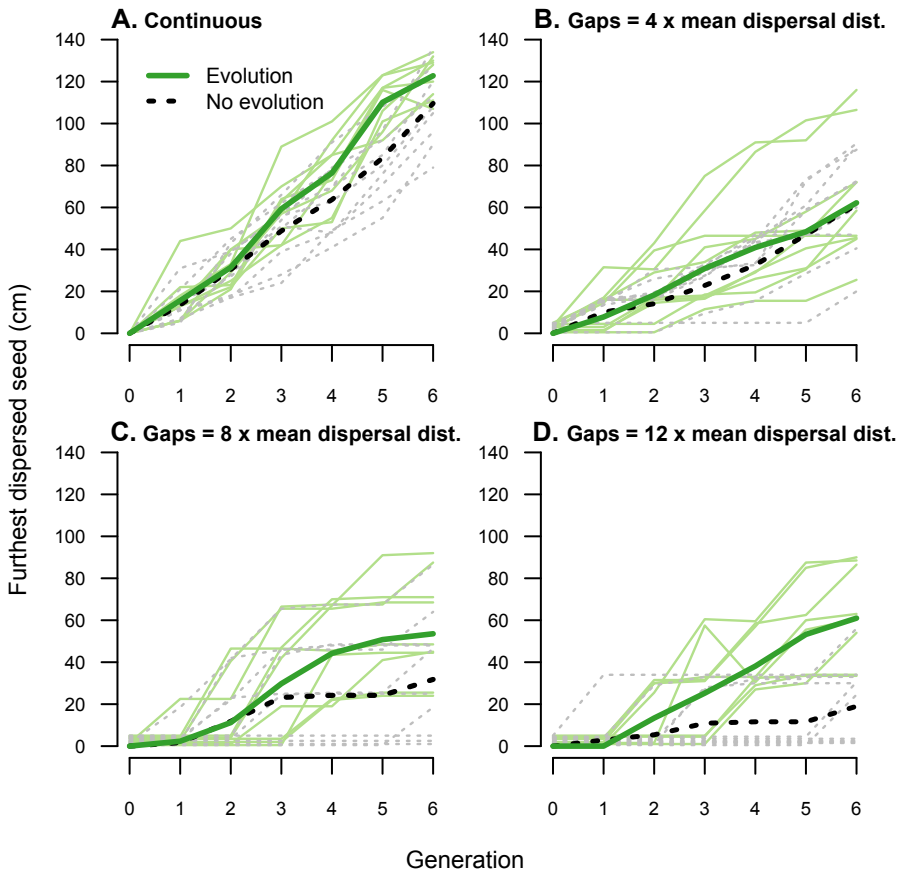

Fig. 2. Furthest distance colonized in each generation
Distance moved by evolving (thin green solid lines) and non-evolving (thin grey dashed lines) replicate invasions and their mean values (thick green and black lines) in landscapes that are A. continuous, or separated by gaps that are B. 4 × mean dispersal distance, C. 8 × mean dispersal distance, and D. 12 × mean dispersal distance. Lines in the three patchy landscapes are jittered for visibility.

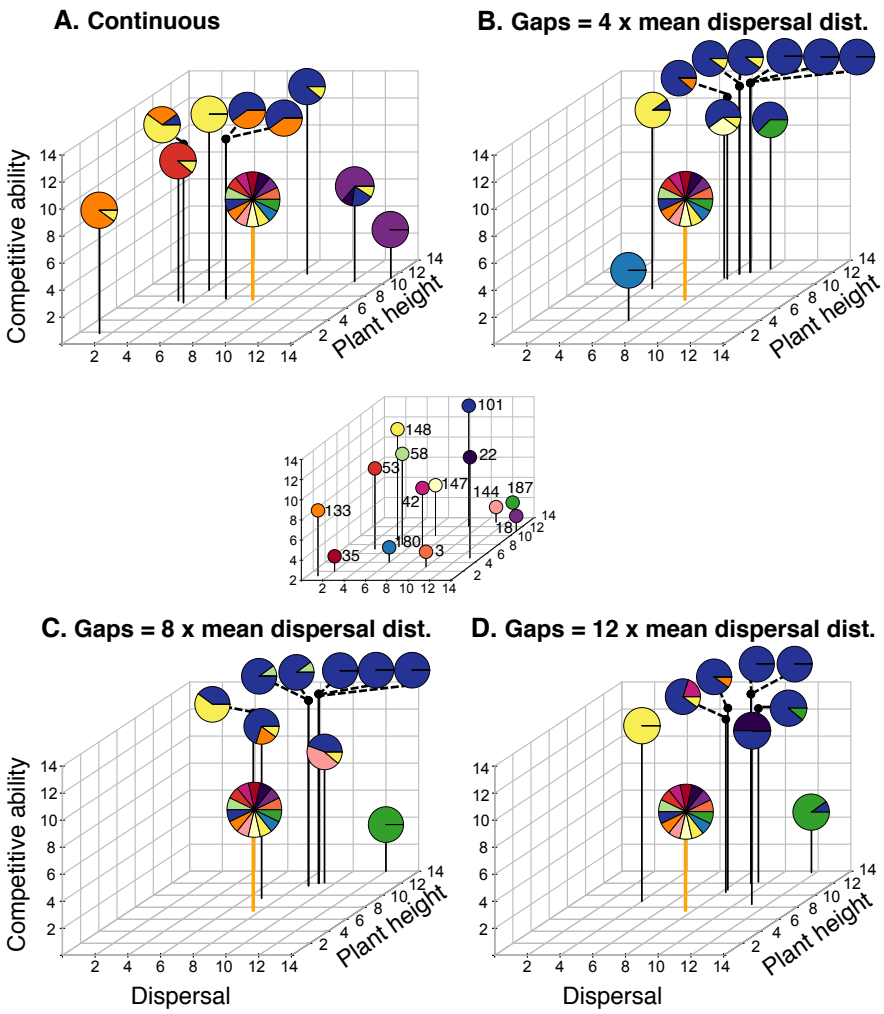

Fig. 3. Genotypes and traits at the invasion fronts

The central pinwheel of each panel depicts the equal frequency of genotypes in the founding population, and is located at the mean trait rank for three spread-relevant traits: competitive ability (dominance in non-spreading context); dispersal (average distance of furthest dispersed seed from a solitary individual); and plant height. Pies show the genotypic composition of the 10 leading individuals for each replicate invasion after 6 generations of spread through landscapes that are A. continuous, or separated by gaps that are B. 4 × mean dispersal distance, C. 8 × mean dispersal distance, and D. 12 × mean dispersal distance. The location of each replicate is given by the genotype-weighted trait rank mean (explained in the Methods). A fourth trait, seed mass, also evolved, but its evolution did not vary with landscape patchiness and is not shown here. Central panel shows trait ranks of the 14 genotypes; numbers indicate genotype identity.

Table 1. Evolution of spread-relevant traits as a function of landscape patchiness

Results of linear models examining the change in height, dispersal, competitive ability, and seed mass after 6 generations of evolution as a function of landscape patchiness (size of gaps between suitable habitat). Trait change was measured as the difference between the genotype-weighted trait rank for each replicate ($N = 36$) and 7.5 – the mean trait rank of 14 genotypes in the founding population. Significant slopes indicate that the amount of change in the trait increased with increasing gap size (units of mean dispersal distance). Significant intercepts indicate that the trait changed significantly from the founding population, even in continuous landscapes. For competitive ability and seed mass, two traits with non-significant slopes, zero-slope models gave highly significant intercepts ($P \leq 0.001$).

Change in genotype weighted trait rank of:	Intercept			Slope		
	Est.	*t*	*P*	Est.	*t*	*P*
Plant height	1.45	2.25	0.031	0.25	2.83	0.008
Dispersal	-0.64	-0.88	0.386	0.19	1.95	0.060
Competitive ability	3.36	3.74	<0.001	0.15	1.17	0.250
Seed mass	1.29	2.25	0.031	-0.019	-0.24	0.814

List of Supplementary Materials:

Materials and Methods

Figures S1 – S4

Tables S1 – S3

References (30 – 43)

Supplementary Materials for

Rapid evolution accelerates plant population spread in fragmented experimental landscapes

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This PDF file includes:

Materials and Methods

Study system

We developed a laboratory system to study evolution during spread with the model annual plant, thale cress, *Arabidopsis thaliana*. This species is well suited for studying spread, because it completes 3 – 4 generations per year in the greenhouse, allowing us to quantify spread at a rate not possible in the field. Moreover, it passively disperses its seeds on a scale small enough to be compatible with spread in a greenhouse chamber.

To assemble our populations, we selected 14 genotypes from the 162 recombinant inbred lines (RILs) that have been derived from the Cvi and L*er* ecotypes (*30, 31*). From this full set of lines, we initially grew 68 with the *erecta* mutation. This mutation makes plants shorter and sturdier, and thus more amenable for both growth and dispersal in the greenhouse. We further narrowed the set of RILs for the experiment by only considering lines that were 17 – 24 cm tall, and then choosing 14 lines that evenly spanned the range of seed size (13.7 – 32.8 μg), a trait that might correlate with those controlling spread (*32-35*). When multiple RILs had similar seed size, we randomly selected the line to include. As in previous studies, the 14 lines (referred to as genotypes hereafter) can be considered clones, because the selfing rate is > 95% and thus offspring are nearly always identical to the parent (*23, 24*). This property is useful for predicting average population traits from those of the genotypes.

Experimental design

We evaluated the effects of evolution and landscape patchiness on population spread with a factorial application of two treatments, one manipulating the response to selection and the other varying the degree of landscape patchiness. Each experimental unit consisted of a linear array of plastic planting pots of size 21 × 7.3 × 5.5 cm (Bachmann Plantec) oriented in series such that each array was 21 cm wide, and each unit of habitat 7.3 cm long. The total length of the array expanded as the populations spread, but arrays always extended 58.4 cm (8 pots, including gaps) ahead of the last pot containing reproducing individuals. This distance is 24 cm past the maximum dispersal distance observed in preliminary experiments.

We began the experiment at generation 0 by haphazardly sowing 42 seeds (3 seeds of each of the 14 RILs) on the soil surface of the left-most pot of each replicate array. In each generation, seeds germinated, plants grew until fully flowering, and then pots were arranged in linear arrays, where seeds developed and dispersed. In total, we followed each experimental unit for 6 generations of spread (7 generations in total). In 40 of the 80 total replicates, we allowed natural selection to operate—that is the genotypes sorted through space, and frequencies changed in time with no manipulation ('evolution' treatment). In the other 40, we prevented an evolutionary response to phenotypic selection by replacing newly germinated seedlings with individuals drawn with equal probability from each of the 14 genotypes, as began the experiment ('no evolution' treatment, described in more detail below). Although the sampling of the 14 genotypes allows for one generation of drift in each generation, only in the evolution treatment is drift given the opportunity to propagate between generations. We manipulated habitat

patchiness by separating individual pots by gaps that were 0 (continuous landscape), or approximately 4, 8, or 12 times the mean dispersal distance in length (0, 7.3, 14.6, and 21.9 cm, respectively). Ten replicates of each evolution treatment were assigned to each of the four landscape patchiness treatments. Four replicates were discarded due to contamination by genotypes not part of this experiment.

The growing conditions for each generation were as follows. Plants were grown in a 4:1 mix of peat soil (Klasmann Substrat 1, Bigler Samen AG) and sand (Quarzsand N, Carlo Bernasconi AG). To promote simultaneous germination, pots with seeds were watered and placed in a cold room (4 °C) for four days. The initial watering included a treatment of SolBac (Andermatt Biocontrol AG) to minimize growth of fungus gnats. Pots were then moved to two greenhouse chambers set to the following conditions: 19 – 23°C, 60% humidity, 16 hours of daylight, and overhead lights turned on when light fell below 15kLx. Seedlings were watered 2 times per week using flooding and draining tables. Watering frequency increased to 4 times per week once flowering and seed production began. After germination, plants were fertilized with 0.8 g Tardit Langzeitdünger (Hauert), and a 1 × 1 cm hardware cloth mesh was fixed over the pots 6 cm above the soil surface. The mesh stabilized the plants as they grew. To stabilize solitary plants, we loosely tied them to thin wooden stakes. Greenhouse pests were controlled as they emerged during the experiment with a combination of pesticides and biocontrol agents.

Once all plants flowered and began to set seeds (6 – 7 weeks after germination), but siliques (fruits) were still green, pots were moved into new chambers and arranged in the experimental arrays. Each replicate array was separated by a barrier of polypropylene garden fabric (Pflanz-Folie SUPERGROW Frühbeet Vlies, Windhager AG) to prevent seed dispersal between arrays. Humidity was reduced to promote the ripening and opening of siliques. The plants were given six weeks to drop their seeds unassisted. At this point, we simulated one rainfall event with a spray bottle of water positioned 60 cm above the pot surface, aimed to spray directly downward. Each pot, including those ahead of the invasion, receiving four sprays. Wetting and drying of siliques as well as the physical force of the water contacting siliques are important to *Arabidopsis* dispersal. Only seeds that fell within the linear array could contribute to future population growth and spread.

One week following the simulated rain event, pots with no previous occupants were covered, and then removed from the chambers to prevent contamination or human facilitated dispersal. Pots with (now senesced) plants were removed one at a time. The senesced plants were clipped at the soil surface and disposed, and seeds on the soil surface were gently scraped onto a sheet of paper. The scraped material was sieved to remove larger debris, and the seeds were scattered on the surface of a new pot with fresh bare soil. This procedure ensured that soil nutrients did not differ between pots containing their first generation of seeds and those with prior plant occupants. Seeds were allowed to ripen for a further two weeks before the replicate arrays, including all pots with no prior occupants (and therefore not needing to be scraped and transferred), were watered and

placed in the cold room. Each generation (from germinating seed to germinating seed) was approximately 100 days.

To synchronize the two evolution treatments in time, we germinated the 'no evolution' treatment arrays before the 'evolution' treatment because the former needed to have their seeds/seedlings replaced before the next generation began. After the initial germination of the 'no evolution' replicates, seedling numbers were assessed in each pot, and a series of replacement pots mirroring the densities of the original pots were created. We used different approaches to replace individuals in high- versus low-density 'no evolution' pots, because we wanted to ensure that our manipulation did not alter the spatial location of individuals within each low-density pot (high-density pots tended to form a carpet of individuals so their spatial location was not of concern).

For pots with more than 20 seedlings, replacement pots were sown with the same number of seeds as seedlings counted in the original pot, but the seeds were taken from a pool composed of equal frequency of the 14 genotypes (as began the experiment). Germination rates were > 95% so the total number of new seedlings would not differ significantly from the number that were counted. These newly sown pots were then placed in the cold room at the same time as all pots from the evolution treatment, so the two treatments would grow synchronously. For replacement pots with fewer than 20 seedlings, seedlings (not seeds) haphazardly chosen from a seedling tray with an equal mix of all genotypes were transplanted into a new pot in the same location as each seedling in the original pot. These pots were followed closely, so that if a transplant died, it could be replaced immediately, although this happened rarely since transplant success was nearly 100%. Seedling replacement was done after the higher density pots and the 'evolution' pots had germinated, so that all pots were synchronized for growth.

In all treatments, the number of individuals in all pots, and the location of the individuals within the leading pots, were recorded after germination in each generation.

Estimation of genotype-specific parameters

To estimate the fecundity of each genotype, we grew each genotype alone and with 4 densities of neighbors: 14 individuals (1 individual/genotype), 28 individuals (2/genotype), 100 individuals, and 500 individuals, with 8 replicates of each density/genotype. We used the same pots, soil and growing conditions as described above. After plants set seed, we recorded the height and silique (fruit) number of each target individual. From each target individual, we also harvested 5 siliques, from which we counted and weighed seeds (for assessing total fecundity and seed mass).

We investigated dispersal of each genotype with 6 plants per genotype, grown one per pot. Once seeds began to mature, each plant was set up in a dispersal array, with a 21 × 50 cm board covered with sticky backed plastic film extending in one direction from the edge of the pot with the parent plant. Plants were given the same opportunity to disperse as in the main experiment, including the spray treatment described above, and then all seeds on the sticky paper were counted in 1 cm wide bins. Mature plants were clipped, pots scraped, and the scraped seeds were germinated to estimate how many seeds

fell into the parental pot. To estimate dispersal, log-normal dispersal kernels were fit to the seed dispersal distances measured on the sticky plastic film (log-normal kernels fit better than exponential kernels for almost all genotypes) with genotype specific parameters. The height and silique number of parental plants were measured and added to the fecundity and height data described in the prior paragraph to increase the sample size for solitary individuals.

We calculated genotype-specific dispersal as the average distance of the furthest dispersed seed, using Clark et al.'s (*36*) "invasion by extremes" model. The average distance of the furthest dispersed seed is useful because it combines into a single value the several factors determining how far a parent will spread its offspring. Specifically, we determined this value by parameterizing the Clark et al. model with the probability of dispersing beyond the parental pot, the dispersal kernel conditioned on dispersing beyond the parental pot, and the net reproduction of an isolated individual. The net reproduction of an isolated individual was calculated by fitting genotype-specific logistic regression and Gompertz models to the rate at which survival and seed production (conditioned on survival) declined with increasing neighbour density, and using the predicted intercept as the fecundity of an isolated individual. This fecundity was corrected for the germinability of the seeds, such that fecundity indicated the number of germinable seeds produced.

The mean height of solitary individuals was determined by the predicted intercept of genotype-specific linear models describing the rate at which height declined with increasing neighbour density. Although in the paper, we only present the evolution of height when solitary, we also checked how height when crowded evolved. Plants do get shorter when crowded, but all analyses suggested that height when crowded did not evolve differently in landscapes of differing gap size. Thus, we focus our analyses on height when solitary, as it seems to be under differential selection in landscapes of differing patchiness (Fig. 3).

Genotyping

At the beginning of Generation 6, we collected ~20 mg leaf tissue for genotyping from the 10 leading individuals at the front and 10 haphazardly chosen individuals from the left-most (back) pot in each evolving population replicate (800 individuals in total).

Genomic DNA was extracted from lyophilized leaf tissue using a modified CTAB protocol (*37*). Of nine molecular markers previously screened to differentiate between Cvi-L*er* RILs (*24*), seven were able to differentiate the 14 RILs used in this study (for marker details, see Table S2). Amplification was conducted in a two-step Polymerase Chain Reaction (PCR), described by (*38*), in either a multiplex (At01, At05, At06, At07, At09) or simplex (At02, At08) reaction (for PCR details, see Table S3). In the second step, the sequence M13(-21)_FAM was added to all primers as a labeling primer for easy detection by the genetic analyzer. Fragment lengths were determined using either gel electrophoresis (for simplex At08), or capillary electrophoresis on a 3730xl Genetic Analyser (Applied Biosystems, ABI) and using the software Geneious 6.1.8 (*39*), and then manually verified. Identification of the RILs could be done in 97% of all samples. For the remaining 3%, either DNA extractions or PCR reactions failed, or the allele

combination did not match to any of the RILs used in this study. Genetic data produced and analyzed in this paper were generated in collaboration with the Genetic Diversity Centre (GDC), ETH Zurich.

Statistical analysis

To evaluate how the effect of evolution on invasion speed depended on the patchiness of the landscape, we fit a linear model predicting the distance of the furthest individual after 6 generations of spread as function of gap size (as a continuous variable), evolution (as a categorical variable), and their interaction. We analyzed the log transformed distance of the furthest individual so that our model evaluated multiplicative effects of the predictor variables. In addition, for each gap size, we separately compared the distance of the furthest individual (log-transformed) in the evolution and no evolution treatments using *t*-tests and assuming unequal variance. To test whether spread in each evolution treatment differed with gap size in the three patchy landscape types, we fit a separate linear model to these data.

To investigate the patterns of among-replicate variation in invasion speed, we calculated the coefficient of variation (CV) of the distance of the furthest individual. Although CVs are asymptotically F-distributed, our relatively small replication number led us to use bootstrapping to generate confidence intervals for the CV estimates; in particular, we calculated accelerated bootstrap confidence intervals (BC_a) in the `boot` package using R = 9999 replicates (*40, 41*). We calculated pairwise *P*-values (comparing evolution vs. no evolution for each level of landscape patchiness) from the BC_a confidence intervals of the difference between the CVs.

To investigate the amount of trait evolution, for each replicate population front, we calculated a genotype-weighted rank for each of the following four spread-relevant traits: plant height, seed size, dispersal, and competitive ability. We estimated competitive ability as the ranked relative abundance of each genotype after six generations in the non-spreading environment of the left-most pot, from which the invasion originated (after pooling data across all evolving replicates). We used ranks for all four traits, because especially for dispersal and competitive ability, the ranks are more likely than the quantitative differences between genotypes to hold under the crowded conditions of the experiment, and ranking removes the skew in the trait distribution of the genotypes. For example, while the quantitative measure of a genotype's relative abundance in the left-most pot (competitive ability) changes with time, generating an increasingly skewed distribution of genotype competitive ability, the ranks do not have this property. Similarly, absolute differences between genotypes in their dispersal in a non-competitive environment are likely to be compressed in the more competitive environments of the experimental invasions. Finally, by also ranking height and seed size, we could investigate differences in multidimensional trait space between treatments with all traits scaled the same way, which is essential for calculating Euclidean distances (see below). For each replicate invasion front, and each trait, we therefore calculated a genotype-weighted trait rank mean (analogous to a community-weighted trait mean in the community ecology literature).

For each replicate invasion front, we used Euclidean distances to quantify the 14-dimensional genotypic change (14 RILs) and four-dimensional trait change (4 traits) from the founding population. Specifically, we calculated the Euclidean distance for each replicate as the distance between the genotype frequency after six generations and the genotype frequency of the founding population (equal frequency of the 14 genotypes). For the change in trait space, we calculated the Euclidean distance between the genotype-weighted rank of the four spread-relevant traits and the equivalent trait rank of the founding population (mean rank = 7.5 for all traits). We then fit linear models to test if the amount of genotypic or trait change (quantified with Euclidean distance) depended on gap size (significant slope of model). In cases where the slope was not significant, we also fit an intercept-only model to evaluate whether the evolutionary change (across all gap sizes) was significantly different than zero.

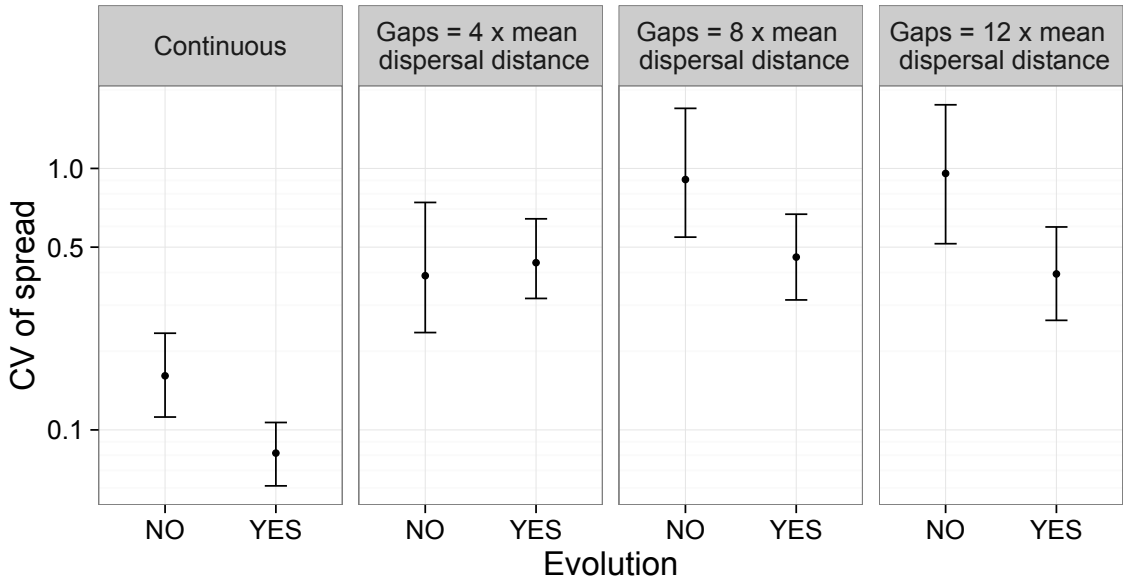
To more specifically explore how each of the four traits evolved during the course of the experiment, we used linear models to test how patchiness (gap size) affected the change in the genotype-weighted trait rank from the founding population. Again, if traits evolved more with increasing gap size, we expected a significant positive slope, and if the traits evolved significantly from the founding population in the continuous arrays, we expected to see a significantly positive intercept in the model (or in an intercept-only model). We used a distance-matrix based analysis of variance analogous to MANOVA (R package adonis {vegan} (*42*)) to explore how the genotypic composition of the leading individuals in each replicate varied with increasing patchiness. This permutation-based approach analyzed the Euclidean distance between replicate invasion fronts in their genotypic composition and involved 100,000 permutations.

Finally, for all landscape types, we compared the observed among replicate variation in the genotypic composition of leading individuals to that expected by randomly sampling from the total pool of genotypes at the invasion front (for a given level of patchiness). For each patchiness treatment, we first defined the average proportional composition of all genotypes at the invasion front by summing over the 10 leading individuals in all replicates. We then calculated the Euclidean distance between this average composition and the genotypic composition of each replicate invasion front (ten individuals), and then summed over all replicates. This provides a measure of the compositional variation across replicates within a patchiness treatment. We then compared this measure of compositional variation to that expected by chance sampling from the total pool of leading individuals. Specifically, we simulated the genotypic composition of 8-10 replicate invasion fronts (equal to the number of evolving replicates in the patchiness treatment) each composed of 10 individuals randomly drawn with replacement from the total pool of leading individuals (for that landscape type). We then calculated the Euclidean distance between each of the replicates and the average genotypic composition of the replicate invasion fronts, and summed those distances to give a measure of among replicate variation expected under random sampling. By repeating this simulation 100,000 times, we estimated the probability of obtaining the observed among-replicate compositional variation based on random sampling alone. All analyses were conducted in R (*43*).

Simulations of spread velocity

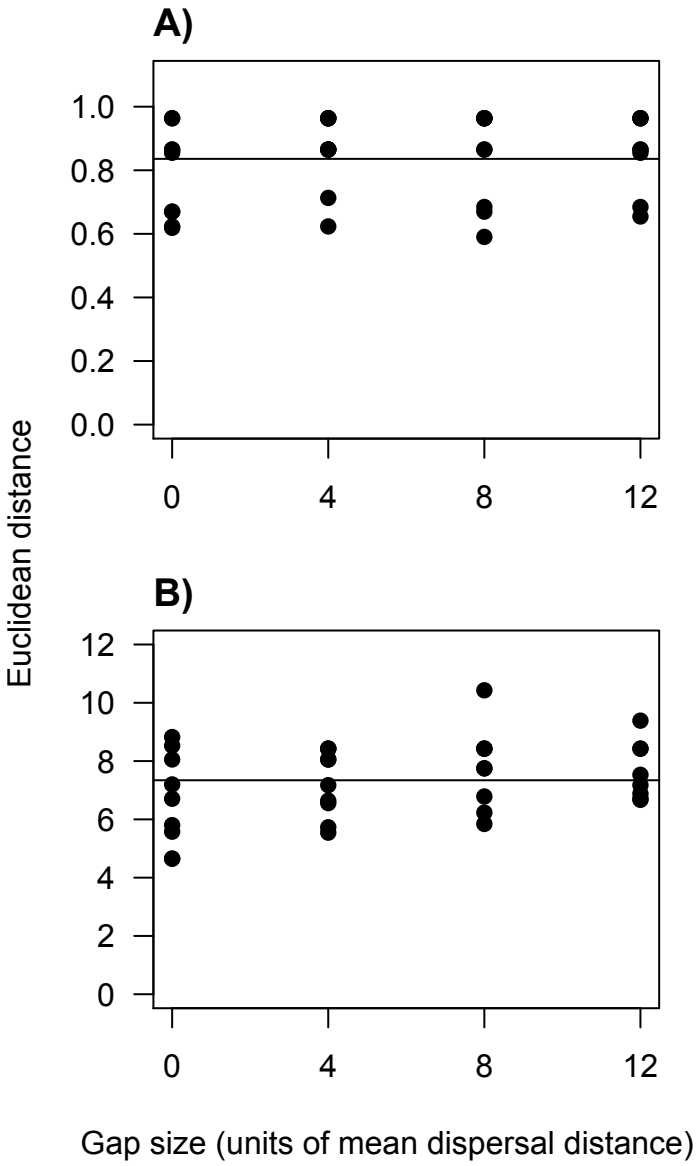
We conducted simulations to evaluate how competitive ability affects the spread velocity of an annual plant (like *A. thaliana*) moving through continuous and fragmented landscapes. These simulations match those of reference (*11*) with the addition of Poisson distributed demographic stochasticity in seed production. As with the experiments presented here, the simulations involved an annual plant population spreading through a linear array of suitable, discrete habitat patches that could be separated by gaps, with seeds falling into gaps dying. Density-dependent population growth was modeled by a Beverton–Holt function determined by low-density fecundity and sensitivity to competition (α). Populations composed of genotypes with low sensitivity to competition (low α), were more competitive, and made more offspring under crowded conditions. Individuals dispersed following a double-sided negative exponential kernel. Model parameters were based on our measurements of *A. thaliana* in the experiments and produced spread velocities in continuous landscapes (those with gap size = 0) that matched those observed in the experiment. Our efforts to fit genotype-specific α's that predict the observed competitive dynamics were unsuccessful, so we varied α over a range of values that produced population densities comparable to those observed in the experiments. Simulations were run for 500 generations, except for $\alpha \geq 0.7$ in simulations in fragmented landscapes, which were run for 10,000 generations (to overcome large stochastic variation). Velocity was calculated from generation 20 onwards to allow the invasion front to first develop. Simulations were run in R (*43*). See reference (*11*) for further details.

Fig. S1



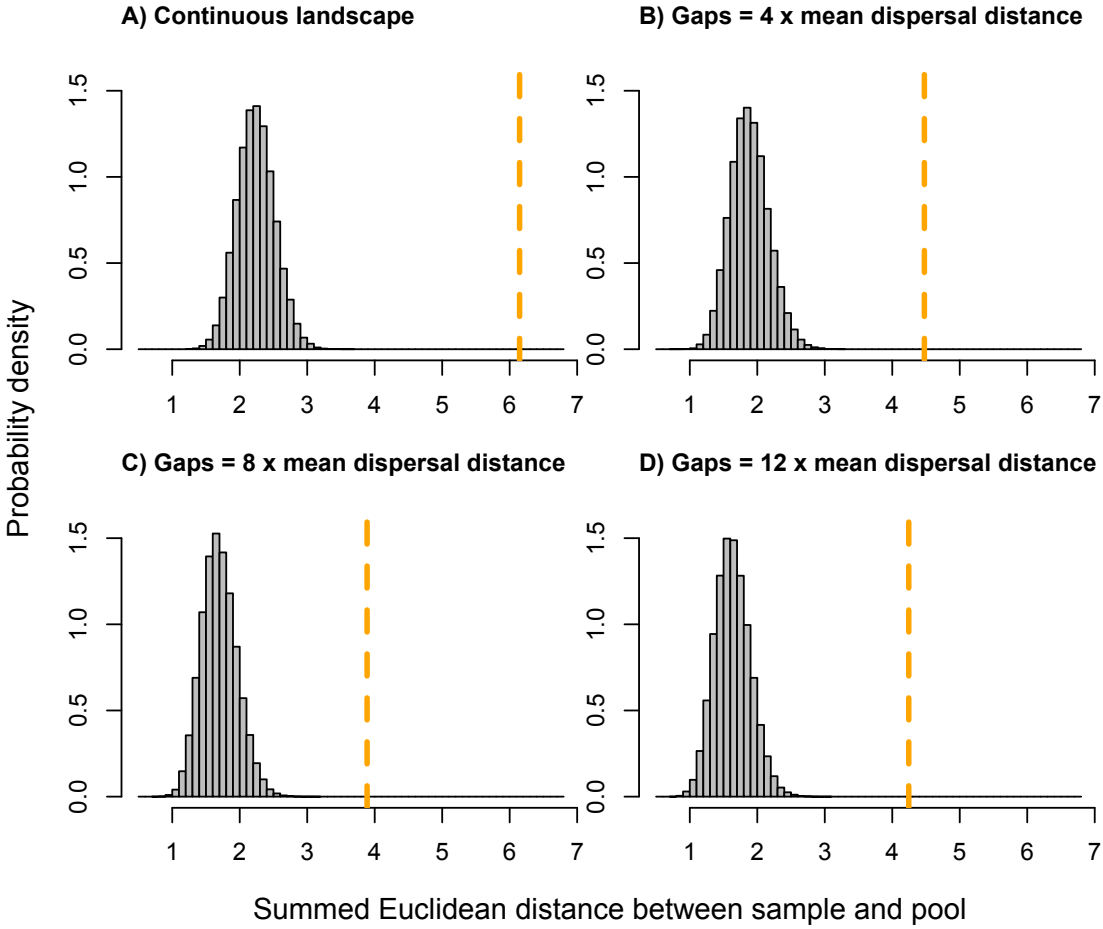
Coefficient of variation of furthest distance colonized after six generations of spread for replicates in evolving and non-evolving populations at each gap size. Points show the observed CVs; error bars show the bootstrapped 95% BC_a confidence intervals, based on 9999 bootstrap replicates. Within each landscape, the bootstrapped 2-sided *P* values testing the null hypothesis that the CVs were equal between the two evolution treatments were 0.01, 0.7, 0.02, and 0.02, respectively.

Fig. S2



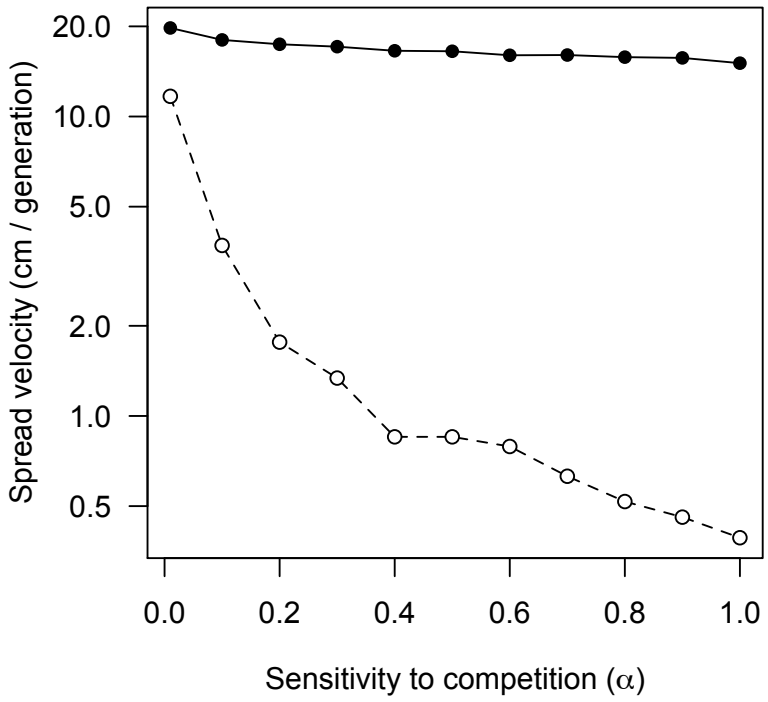
Effects of gap size on the amount of genotypic and trait change after six generations of spread. A) Change in genotype composition from a founding population with equal frequencies of the 14 genotypes. B) Change in trait space from the founding population based on the genotype-weighted rank of four traits influencing spread: the average distance of furthest dispersed seed; competitive ability (dominance in non-spreading context); plant height, and seed mass. The founding population possessed the mean rank (7.5) for each trait. Horizontal lines indicate a significant intercept and non-significant slope to the relationship between genotypic or trait change and gap size (Table S1). The plotted lines are those from a subsequent intercept-only model.

Fig. S3



Observed variation in genotypic composition among replicates in relation to that expected via chance sampling from the total pool of leading individuals for landscapes that are A) continuous ($N = 9$); or separated by B) gaps = 4 × mean dispersal distance ($N = 10$); C) 8 × mean dispersal distance ($N = 9$); and D) 12 × mean dispersal distance ($N = 8$). Grey bars represent the expected distribution of summed Euclidean distances by chance sampling and the dashed orange line represents the observed summed Euclidean distance. For all landscape types, the observed summed distances arise with a probability $P < 0.001$ from chance sampling of the leading individuals of all replicates. For details on the procedure, see Statistical Analysis.

Fig. S4



Simulation results showing that the effect of increasing competitive ability (lower α) on spread velocity is larger in patchy landscapes (open circles, dashed line) than in continuously favourable landscapes (closed circles, solid line). This effect emerges because populations in landscapes fragmented by large gaps tend to build up before they advance, creating a strong influence of competition on the spread velocity (in our experiment, leading edge patches in the most fragmented landscapes had densities >100 individuals 52% of the time). Though the effects of increased competitive ability are much weaker in continuously favourable landscapes (where leading edge patches never had densities > 100 individuals in our experiment, and 93% of the time had 10 or fewer individuals), these effects remain significant because crowded individuals behind the front can occasionally contribute to the expansion. For example, the figure shows that a reduction in α for a population from 1.0 to 0.3 is sufficient to give the 11% increase in spread velocity observed with evolutionary change in the continuous landscapes of our experiment. Model parameters included an exponential decay parameter for the dispersal kernel of 2.5, low-density fecundity of 200, and for the fragmented landscape, a gap size of 8 × mean dispersal distance. All parameters were selected to generate dynamics comparable to those of the populations in the experiments. Simulation procedures are described in the Methods.

Table S1.

Effect of gap size on evolutionary divergence of populations from the founding population in genotypic space and trait space. Divergence measured by the Euclidean distance between each replicate and the founding population with equal frequencies of the fourteen genotypes (genotypic space), or from the trait space defined by dispersal, competitive ability, seed size and height (all based on genotype-weighted trait ranks). Given the positive fitted slopes, significant intercepts indicate significant evolutionary divergence from the founding population. Significant slopes indicate that divergence from the founding population increased with gap size. In both genotypic and trait space, an intercept-only model also gave a highly significant positive intercept ($P < 0.001$), whose value is displayed in fig. S2.

	Genotypic space			Trait space		
	Estimate	*t*	*P*	Estimate	*t*	*P*
Intercept	0.813	22.42	<0.001	6.836	19.34	<0.001
Slope	0.004	0.81	0.426	0.087	1.79	0.082

Table S2.

Summary of the markers, PCR primers and resulting fragment length polymorphism for genotyping (following ref. *24*). Note that the sequence M13(-21)_FAM (TGTAAAACGACGGCCAGT) was added to all primers in the second step for detection in the ABI.

Cereon/TAIR Published Name	Reference Name	PCR primer 1 (5' -> 3')	PCR primer 2 (5' -> 3')	Expected Ler PCR product size (bp)	Expected Cvi PCR product size (bp)
470095	At01	CAATAGAATTTGGCTGCCGTGCCA	ATTACGTGCCTCTCTTGTCCGCTA	234	278
458557	At02	GTCCTGGAGATGGTGGACAG	GGCAAAACCCTAATGTGGAA	405	651
464890	At05	TTGCCTCTGTGGCTGCTACTGAAT	AGTTGACCTCACACACTGAGCCAT	87	144
473983	At06	GTTGTCAACATTCAGGTAACCAC	GTACAATGCTCATGCCTTCTCC	173	237
nga6	At07	ATGGAGAAGCTTACACTGATC	TGGATTTCTTCCTCTCTTCAC	136	166
G3883-1.4	At08	TGTTTCAGAGTAGCCAATTC	CATCCATCAAACAAACTCC	700	1363
457148	At09	CACATCTGAAGCTGTGTTGCTCGT	CGCTAACGCTCTTTGGCGATCTTT	410	523

Table S3.

Details of PCR conditions. M13 refers to the sequence M13(-21)_FAM, which was added to all primers in the second step of the PCR for detection in the ABI.

Multiplex/Simplex:			**MP1**			**MP2**			**At02**			**At08**	
T_A (^{o}C):				60			60			60			60
	Stock [ ]			**1X (µl)**			**1X (µl)**			**1X (µl)**			**1X (µl)**
H_2O				13.6			14.8			16.1			16.7
Buffer (X)	1			5			5			5			5
dNTPs (mM)	10			0.5			0.5			0.5			0.5
Primer F - M13 (µM)	10		At01	0.1		At05	0.1		At02	0.2		At08	0.3
Primer R (µM)	10		At01	0.4		At05	0.4		At02	0.5		At08	0.3
Primer F - M13 (µM)	10		At07	0.4		At06	0.3			0			0
Primer R (µM)	10		At07	1		At06	0.9			0			0
Primer F - M13 (µM)	10		At09	0.2			0			0			0
Primer R (µM)	10		At09	0.8			0			0			0
M13-tail (µM)	10			0.8			0.8			0.5			0
Taq polymerase (U/ µl)	5			0.2			0.2			0.2			0.2
Total rxn volume (µl):				23			23			23			23
DNA template (µl):				2			2			2			2
Thermocycler Conditions			**T (^{o}C)**	**t (sec)**		**T (^{o}C)**	**t (sec)**		**T (^{o}C)**	**t (sec)**		**T (^{o}C)**	**t (sec)**
Initial denaturation			94	120		94	120		94	120		94	120
Denaturation			94	30		94	30		94	30		94	30
Annealing	**35 X**	TD	60	60	TD	60	60		60	60		60	60
Extension			65	45		72	45		72	120		72	120
M13 Denaturation			94	30		94	30		94	30			
M13 Annealing	**8 X**		53	45		53	45		53	45			
M13 Extension			72	45		72	45		72	45			
Final extension			72	600		72	600		72	600		72	600