Reciprocal transplants demonstrate strong adaptive differentiation of the model organism *Arabidopsis thaliana* in its native range

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Summary

- To quantify adaptive differentiation in the model plant *Arabidopsis thaliana*, we conducted reciprocal transplant experiments for five years between two European populations, one near the northern edge of the native range (Sweden) and one near the southern edge (Italy).
- We planted seeds (years 1–3) and seedlings (years 4–5), and estimated fitness as the number of fruits produced per seed or seedling planted.
- In eight of the 10 possible site × year comparisons, the fitness of the local population was significantly higher than that of the nonlocal population (3.1–22.2 times higher at the southern site, and 1.7–3.6 times higher at the northern site); in the remaining two comparisons no significant difference was recorded. At both sites, the local genotype had higher survival than the nonlocal genotype, and at the Italian site, the local genotype also had higher fecundity. Across years, the relative survival of the Italian genotype at the northern site decreased with decreasing winter soil temperature.
- The results provide evidence of strong adaptive differentiation between natural populations of *A. thaliana* and indicate that differences in tolerance to freezing contributed to fitness variation at the northern site. In ongoing work, we explore the functional and genetic basis of this adaptive differentiation.

Introduction

'In all species complexes that cover climatically different areas, the phenomenon of regional differentiation stands as a fundamental principle.' Clausen *et al.* (1940, p. 411)

Perhaps more than any other group of organisms, plants have played a leading role in the study of adaptation. In his 'big book' on natural selection, which served as the template for *The Origin of Species* (Stauffer, 1975), Darwin presented a detailed example of adaptive divergence in plant populations exposed to differing environments (Schemske, 2010). The concept of the 'ecotype', for example, populations of the same species adapted to their local environmental conditions, was first introduced by Turesson (1922) following his experimental studies of natural plant populations. Clausen *et al.* (1940) provided numerous examples of adaptation in plants through comprehensive reciprocal transplant experiments in which populations were planted at or near their original collection sites.

The incidence and strength of local adaptation depend on a number of factors, including the scale and magnitude of spatial variation in selection, the extent of gene flow, and the heritability of adaptive traits. Because these factors differ widely between species and populations, so does the magnitude of local adaptation (Leimu & Fischer, 2008). For example, strong microspatial adaptation is observed in some species (Turkington & Harper, 1979; Schemske, 1984), while in others, adaptation is manifest only at very large scales (Galloway & Fenster, 2000). Furthermore, local adaptation is sometimes strong between species, but weak or nonexistent within species. For example, Angert & Schemske (2005) conducted reciprocal transplants beyond the range margins of two closely related *Mimulus* spp. that occupy different altitudes and found striking fitness differences between species, but no differences among populations within species.

Reciprocal transplant experiments have provided a number of important insights concerning the extent of local adaptation and of the factors contributing to adaptive differentiation in both plants and animals (Kawecki & Ebert, 2004; Leimu & Fischer, 2008; Hereford, 2009). First, local adaptation is common, although not ubiquitous. Leimu & Fischer (2008) found evidence of reciprocal adaptation in 45% of studies on plants, and Hereford (2009) found that 71% of studies of plants and animals gave evidence of local adaptation. Secondly, the magnitude of local advantage is often large. Hereford (2009) found that local

populations of plants and animals have, on average, a 45% advantage over the nonlocal population. Thirdly, small populations may sometimes lack the genetic variation required to adapt to local conditions (Leimu & Fischer, 2008). Fourthly, there is only weak evidence for adaptive tradeoffs (Hereford, 2009). Finally, there is a significant, positive association between fitness differences and the magnitude of environmental differences between parental sites (Hereford, 2009). This, plus the lack of correlation between the magnitude of local adaptation and geographic scale (Leimu & Fischer, 2008), demonstrates a clear role for ecological factors as drivers of adaptive differentiation.

To understand the ecological and genetic mechanisms of adaptation requires comprehensive ecological and genetic studies identifying the phenotypic traits subject to selection, the ecological factors that cause selection (MacColl, 2011), and the genetic architecture of adaptive traits. Reciprocal transplant experiments can be used to quantify the degree of adaptive differentiation, but also to identify the life history stages at which adaptive differences are expressed, and thereby suggest candidate adaptive traits. The adaptive significance of putative adaptive traits can be examined through experimental manipulation of trait expression (e.g. Sletvold & Ågren, 2011), while selective agents can be identified through manipulation of the abiotic or biotic environment (Sandring & Ågren, 2009). Finally, through genetic studies, the genomic regions that harbor genes responsible for adaptive differentiation can be revealed (Lowry et al., 2009). Studies of this kind ultimately offer the opportunity to link the ecological factors that contribute to divergent natural selection to the genetic mechanism of adaptive evolution.

Model organisms provide exceptional opportunities to investigate the functional and genetic mechanisms of adaptation, yet we know very little about natural populations of these species in their native environments. Arabidopsis thaliana (hereafter Arabidopsis) has become the workhorse of plant biologists worldwide, owing to its small size, selfing habit, annual life history, and the extraordinary genetic resources that have been developed for its study. Major advances in the fields of molecular and cellular biology, plant physiology, developmental genetics, and genomics were catalyzed by research on Arabidopsis (Redei, 1992; Meyerowitz, 2001), yet relatively few studies have examined adaptation in natural Arabidopsis populations. This is perhaps surprising, given that collections of Arabidopsis are still frequently referred to as ecotypes (but see Pigliucci, 1998; Alonso-Blanco & Koornneef, 2000).

Some 60 yr ago, Laibach (1951) proposed that life history differences between Arabidopsis populations probably represent an adaptive response to different winter temperature regimes, and one decade later, Ratcliffe (1961) described the after-ripening requirement of seeds as an adaptation to delay germination in populations that experience hot, dry summers. Since these early efforts, there has been increasing interest in using Arabidopsis as a model system for exploring the ecological and genetic mechanisms of adaptation. Many different approaches have been employed, including association mapping (Atwell et al., 2010; Bergelson & Roux, 2010; Fournier-Level et al., 2011; Hancock et al., 2011), quantitative trait locus (QTL) mapping (Weinig

et al., 2003; Huang et al., 2010), studies of latitudinal or altitudinal clines (Maloof et al., 2001; Stenøien et al., 2002; Michael et al., 2003; Stinchcombe et al., 2004; Hannah et al., 2006; Zhen & Ungerer, 2008; Montesinos-Navarro et al., 2011), common gardens (Griffith et al., 2004; Rutter & Fenster, 2007) and reciprocal transplant experiments (Callahan & Pigliucci, 2002; Arany et al., 2008). Mitchell-Olds & Schmitt (2006) discussed the advantages and disadvantages of these and other approaches to the study of adaptation in Arabidopsis, and concluded (p. 951) that:

'… direct support for the local adaptation hypothesis requires reciprocal transplants between natural populations, and selective mechanisms are best tested by measuring natural selection on traits of interest in such experiments. Although this approach has proved to be very powerful in other plant species, it has rarely been attempted for A. thaliana, and evidence for local adaptation has been equivocal. A reciprocal-transplant approach could be very valuable for testing hypotheses about local adaptation to climate across the native range.'

Here we report the results of reciprocal transplant experiments conducted over five years with natural populations of Arabidopsis collected from north-central Sweden and central Italy and grown in their native environments (Fig. 1). These localities represent, respectively, the northern and southern limits of the native range (Koornneef et al., 2004), and differ greatly in a number of environmental factors, including temperature and day length. We therefore expect geographic differences in the phenotypes favored at each site.

Fig. 1 Map indicating the locations of the two study sites.

We address the following questions: first, is there evidence of geographic adaptation for overall fitness, such that the local population outperforms the nonlocal population? Secondly, if so, which components of fitness (establishment, winter survival, fecundity) contribute most to the local advantage? Thirdly, how do temperature regimes differ between sites, and what role does temperature play in adaptation? Because low winter temperatures can be assumed to represent a major challenge at the northern site, we asked whether among-year variation in relative survival of the two populations could be related to the minimum soil temperature recorded at the two sites. Finally, because an appropriate response to seasonal cues is critical for plant fitness in seasonal environments we asked: are differences in fecundity associated with genetic differences between populations in flowering time?

This study is the first step towards a comprehensive investigation of the ecological and genetic mechanisms of adaptive differentiation in *Arabidopsis*. Ongoing field and laboratory experiments seek to identify the ecological factors, plant traits and genes that contribute to the fitness differences described here.

Materials and Methods

Study sites and seed collection

To capture major environmental differences that might contribute to climatic adaptation, we chose populations of *Arabidopsis thaliana* (L.) Heynh located near the northern and southern limits of the native geographic range in Europe (see Koornneef *et al.*, 2004); one population from central Italy, Castelnuovo di Porto (42°07′N, 12°29′E; hereafter Castelnuovo), located 24 km north of Rome; and one from north-central Sweden, Rödåsen (62°48′N, 18°12′E), located 64 km northeast of Sundsvall (Fig. 1). The estimated annual precipitation is 817 mm at the Italian site and 653 mm at the Swedish site (http://www.world clim.org/; Hijmans *et al.*, 2005). The two sites have relatively little human disturbance and are representative of the local communities of native plants.

Phylogeographic analysis of haplotypes indicates that these populations are typical of those found within each geographic region (Beck *et al.*, 2008). Both populations grow on steep, rocky slopes near forest, and both are winter annuals. Seeds germinate in the fall (October–December in Italy, August–October in Sweden), and overwinter as rosettes. Plants flower in February–April in Italy and in May–June in Sweden. At the start of flowering, day length is *c.* 10 h at the Italian site and *c.* 16.5 h at the Swedish site (http://www.timeanddate.com/).

Seeds were collected in June 2002 in Sweden and in April 2003 in Italy. To obtain a sufficient pool of seeds for each line and to reduce maternal effects, plants were grown in growth chambers for at least one generation, and seeds were produced by autonomous self-pollination. For the present experiment, we used seeds of one maternal line from each population because preliminary studies indicated very limited within-population variation in fitness and in putative adaptive traits such as tolerance to freezing and flowering time. Tests of tolerance to freezing, which included three maternal lines from each of two Swedish

and two Italian populations (including the Castelnuovo and Rödåsen populations), detected large and statistically highly significant regional differences in freezing tolerance (Italy vs Sweden), but no differences between populations within regions or lines within populations (D. Schemske & J. Ågren, unpublished). In a field experiment that examined the effect of timing of germination on survival and fecundity in the Rödåsen population, and that included eight maternal lines from the local population with 30 replicates per line, no among-line variation in survival, fecundity or total fitness was detected (Akiyama, 2011).

Temperature

To characterize the microclimatic conditions at the two study sites, we recorded air and soil temperature once per h with HOBO Temperature Data Loggers (HOBO Pro Data Logger Series® H08-031-08, Onset Computer Corporation, Bourne, MA, USA) from the autumn of 2003 until the end of the final experiment in spring 2011. We recorded air temperatures *c.* 30 cm above the ground and soil temperatures *c.* 1 cm below the soil surface with two loggers at each site. The soil temperature is probably most relevant to plant growth and survival during winter when plants are in the rosette stage, with prostrate leaves and roots just below the soil surface.

Reciprocal transplant experiments

To assess the magnitude of geographic adaptation, we conducted reciprocal transplant experiments in five years. In a first set of experiments, seeds from the two study populations were reciprocally planted in three consecutive years (2004–2006) in experimental gardens established in natural vegetation within 0.5 km from the source populations. The seeds were planted before or at the time of natural germination in the source populations (in Sweden on 17 August 2004, 27 August 2005, and 20 August 2006; in Italy on 11 September 2004, 12 September 2005, and 4 September 2006). Seeds were planted in 7-cm-wide circular plots arranged in a rectangular array with individual plots separated by 5–10 cm. Plots were delimited by 5-cm-tall PVC cylinders sunk into the ground, leaving *c.* 1 cm of the cylinder above the soil surface. Before planting, the transplant plots were cleared of vegetation and the topsoil was replaced by soil collected nearby under vegetation too dense to provide suitable growing conditions for *Arabidopsis*. In this way, we were able to use local soils without chemical or physical treatment to eliminate native *Arabidopsis* seeds. Planting treatment was randomly assigned to plots (Swedish seeds, *n* = 50 plots; Italian seeds, *n* = 50 plots; control (not planted), *n* = 20 plots). In the 2004/2005 experiment, 30 seeds were planted per experimental plot; in the 2005/2006 and 2006/2007 experiments, 60 seeds were planted per plot. Controls were established to quantify natural seedling establishment. In all three years and at both sites, far fewer plants were observed in control plots compared with those that received seeds, suggesting that the vast majority of plants scored were actually established from experimental seeds (5.2 times as many plants were recorded in experimental plots planted with the genotype

with the lowest seedling establishment compared with controls (median for six site × year combinations)). Any background germination in experimental plots should reduce the proportional difference in performance between local and nonlocal genotypes, and the estimates of population differentiation obtained should thus be conservative.

In a second set of experiments, seedlings originating from the two study populations were reciprocally transplanted in two consecutive years at the same sites as used for the first set of experiments. These trials were part of a large, ongoing experiment conducted with parents and recombinant inbred lines (RILs) to identify QTLs associated with adaptive traits. The results from the QTL-mapping of the RILs will be reported elsewhere.

For the experiments initiated with seedlings, seeds were planted in Petri dishes on agar and stored in the dark at 4°C for 1 wk to synchronize germination. After the initial stratification, the dishes were moved to a growth room (22 : 16°C, 16 h day at 150 μmol m⁻² s⁻¹ photosynthetically active radiation) where the seeds germinated. Nine days later, seedlings were transplanted to randomized positions in plug trays (20 mm × 20 mm × 40 mm) filled with local soil in Italy, and with an equal mixture of local sand, gravel and unfertilized peat in Sweden. Initial analysis of the results from the first year of the seedling experiment indicated marked edge effects, so the final analysis was conducted after excluding plants in the outer three rows of the array, giving a final sample size of c. 150 plants per site × source combination (Italian site, Italian genotype, $n = 150$, Swedish genotype $n = 156$; Swedish site, Italian genotype, $n = 149$, Swedish genotype, $n = 151$). In the 2010/2011 experiment, 180 plants of each genotype were planted in nonedge positions at each site. Edge positions were planted with plants derived from a cross between the two source populations.

During transplantation, plug trays were kept in a glasshouse at c. 18 : 12°C and 16 : 8 h day : night. Within 6 d, the trays were transported to the field sites where they were sunk into the ground (on 16 September 2009 and 10 September 2010 in Sweden, and on 7 November 2009 and 30 October 2010 in Italy). At the time of transfer to the field sites, the experimental plants had developed or had begun developing the second pair of true leaves, and were thus at the same stage of development as naturally germinating plants in the source populations.

We considered three components of fitness: number of seedlings established per planted seed in the first set of experiments, and survival to reproduction and number of fruits per surviving plant in both sets of experiments. Total fitness was quantified as the number of fruits produced per seed or seedling planted, respectively. In the first set of experiments, the unit of observation was the plot and we scored the number of seedlings, vegetative plants and reproductive plants one to three times during the period October–December at both sites; three to five times from January to fruit maturation in April/May in Italy, and three to five times after snowmelt in April until fruit maturation in June in Sweden. For each plot, we estimated seedling establishment per seed planted by dividing the maximum number of plants recorded during the experiment by the number of seeds sown. Survival to reproduction was quantified as the number of

fruit-producing plants in the plot divided by the maximum number of plants recorded. At fruit maturation, the total number of fruits produced in each plot was recorded. From these data, we calculated mean fruit production per reproducing plant and number of fruits produced per seed planted for each plot. In the second set of experiments, we recorded the survival of transplanted seedlings within 1 wk of the transplant to the field. Plants that had not survived this initial phase were not included in subsequent analyses. After the initial census, the status of all transplanted plants (alive/dead) was checked at least once before the end of the year, and the flowering time of individual plants was determined from censuses conducted every 1–5 d during the flowering period. At fruit maturation, we recorded the number of fruits produced by reproducing plants. In total, the fate of 5936 plants was determined and 116 744 fruits were counted during the course of the two sets of experiments.

To determine whether differences in number of seeds per fruit partly compensated for differences in fruit production between genotypes, we sampled up to three mature fruits from each plot in the 2005/2006 experiment, and, when possible, one mature fruit from each fruit-producing plant in the 2010/2011 experiment, and recorded the number of seeds produced per fruit.

Statistical analysis

In both experiments, three-way ANOVA including site, population and year as independent variables (fixed) indicated that the effect of source population on overall fitness varied among years and sites (significant population × year, population × site, and/or site × population × year interactions). We therefore further examined the effects of site and population of origin on overall fitness and individual fitness components with two-way ANOVA and logistic regression, separately by year.

In the first set of experiments, we used two-way ANOVA to examine the effect of site and population on individual fitness components recorded at the plot level (maximum number of seedlings observed per seed planted, proportion of seedlings surviving to fruiting, and mean number of fruits per fruit-producing plant), and on total fitness (mean fruit production per planted seed). To improve normality of residuals and homogeneity of variances, number of seedlings per seed planted, mean number of fruits per survivor, and number of fruits per seed planted were square-root- or log-transformed, and survival was arcsine-square-root-transformed before analyses. When the site × population interaction was statistically significant, contrasts were used to examine differences between the local and nonlocal population separately by site. In the 2004/2005 experiment, seedling establishment was poor and survival very low at the Italian site. As a result, fruiting plants were observed in only 10% of the plots (five plots planted with the Italian population, and five plots planted with the Swedish population). Because of the large number of plots with no fruiting plants, it was not possible to use ANOVA to test the statistical significance of the effect of the site × population interaction on total fruit production per seed planted. Instead, we used one-way ANOVA to determine whether total fitness was higher for the local than for the nonlocal

population at each site. For the Italian site, the analysis was confined to plots with fruiting plants.

In the second set of experiments, the effects of site and population on survival were examined with nominal logistic regression, while effects on number of fruits per survivor and number of fruits per seedling planted were analyzed with ANOVA. The latter two response variables were square-root-transformed before analyses. Because the site × population interaction was statistically significant in all analyses, logistic regression was used to examine differences in survival between local and nonlocal population separately by site, while contrasts were used to examine differences in number of fruits per survivor and number of fruits per seedling planted separately by site.

In the second set of experiments, data on number of fruits produced were available for individual plants, and we therefore also examined the effect of site and population on total fitness using 'aster models', which in a single analysis may model both survival and fecundity based on appropriate statistical distributions (Geyer *et al.*, 2007). When a significant site × population interaction was detected, we proceeded to examine the effect of population separately by site. Because the results of aster modeling were fully consistent with those obtained from ANOVA models of total fitness (results not shown), we only present the ANOVA results here, which are directly comparable to those reported for the first set of experiments.

To obtain an estimate of mean relative fitness of the two populations across the five years of the study, we calculated for each site and year the relative fitness of the two genotypes as mean fitness divided by the mean fitness of the genotype with the highest fitness based on the product of seedling survival and fecundity (the two components of fitness that were recorded in both sets of experiments). Because *Arabidopsis* populations form a seed bank (Lundemo *et al.*, 2009), estimates of relative fitness were weighted by the mean fruit production per seedling for each site × year combination (cf. Turelli *et al.*, 2001).

To determine whether differences in survival between the two populations were related to soil temperature in winter, we regressed the relative survival of the nonlocal genotype (survival nonlocal genotype/survival local genotype) on the minimum soil temperature.

In winter 2010/2011, the population density of voles was very high in the Swedish study population, and considerable damage by voles was revealed by a census of the experiment immediately after snowmelt in April 2011. In the areas of the experiment covered by vole runways, plant mortality was very high (98%) compared withi outside runways (20%). Mortality within runways was nonselective relative to genotype, and estimates of the relative fitness of the Italian and Swedish genotypes were very similar whether or not positions within vole runways were included in the analysis (data not shown). Because vole damage is a regular feature of the studied habitat, we included all plants in the analysis presented in the following sections.

Aster models were examined with the aster package in R 2.14.1 (R Development Core Team, 2012), while all other statistical analyses were performed with JMP, version 5.0.1 (SAS Institute Inc., Cary, NC, USA).

Results

Temperature

Temperature data from loggers located at the field site revealed striking differences between the two localities for both air and soil temperatures (Supporting Information, Fig. S1). The mean annual air and soil temperatures were, respectively, 14.4 and 13.9°C in Italy and 4.9 and 6.2°C in Sweden (data from 7 yr, 2004–2010). Across eight winters (fall 2003 through spring 2011), the median (range) minimum air and soil temperatures were −3.2°C (−5.9–0.3) and 1.2°C (−0.1–4.8) in Italy and −17.1°C (−21.4 to −13.2) and −3.5°C (−11.2 to −1.8) in Sweden. At the Italian site there was never sufficient snow to insulate the soil, and minimum air and soil temperatures were positively correlated ($r = 0.71$, $n = 8$, $P = 0.048$). In Sweden, by contrast, annual variation in snowfall resulted in substantial year-to-year variation in the capacity of snow to insulate the soil against extremes of low temperature, uncoupling variation in minimum air and soil temperatures ($r = -0.11$, $n = 8$, $P = 0.79$). This result implies that soil temperature provides the biologically most meaningful metric of the temperature regime for plants overwintering at the rosette stage.

Perhaps of greatest relevance to the opportunity for adaptive differentiation with respect to temperature is the finding that only once in the 8 yr of study did the soil in Italy reach freezing temperatures, and on this day the temperature was barely freezing (− 0.1°C; Table S1). By contrast, for the five years of our study, the soil was frozen on average (range) 128 d (81–154) at the Swedish site, with soil temperatures reaching lows of −4°C in two years and −6°C in one year (Table S1). A similar pattern was observed for the eight winters of temperature records in Sweden.

Overall fitness

Across the five years, the reciprocal transplant experiments demonstrated a strong advantage to the local population at both sites, and this was true both in the first three years when seeds were planted and in the final two years when seedlings were planted (Fig. 2). The site × population interaction for number of fruits per seed or seedling planted was statistically significant in all years when it was possible to examine this interaction with two-way ANOVA (Tables 1, 2). The strength of the advantage of the local genotype varied among years as indicated by a significant site × population × year interaction in the analysis of the experiments conducted in the second and third years (2005/2006 and 2006/2007; $F_{1,360} = 68.6$, $P < 0.0001$). In the analysis of the experiments conducted in the final two years (2009/2010 and 2010/2011), the site × year ($F_{1,1311} = 292.7$, $P < 0.0001$) and population × year interactions ($F_{1,1311} = 103.9$, $P < 0.0001$) were statistically significant, but not the site × population × year interaction ($F_{1,1311} = 4.9$, $P = 0.30$).

In eight of 10 comparisons, the fitness of the local population was significantly higher than that of the nonlocal population; in the remaining two comparisons no significant difference was
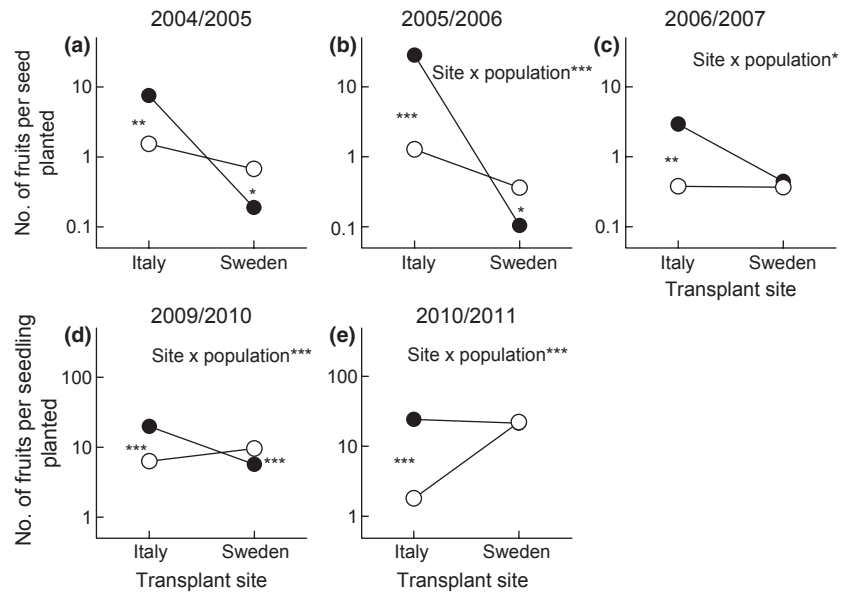
Fig. 2 Fitness of the Italian (closed circles) and the Swedish (open circles) genotypes of *Arabidopsis thaliana* in reciprocal transplant experiments. At both sites, seeds were planted in the first three years, and seedlings were planted in the final two years. Indicated are the results of two-way ANOVA examining the effect of site and population separately by year; contrasts were used to test the effect of population separately by site (except for 2004/2005, when differences were explored with one-way ANOVA separately by site). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 1 Effects of site (Castelnuovo di Porto, Italy vs Rödåsen, Sweden) and population of origin (Castelnuovo di Porto, Italy vs Rödåsen, Sweden) on *Arabidopsis thaliana* seedling establishment, survival to fruiting, number of fruits per survivor, and number of fruits per seed planted in reciprocal transplant experiments conducted in three successive year (2004/2005, 2005/2006, and 2006/2007); analyses conducted on plot means

Source of variation	2004/2005	2005/2006	2006/2007
No. of seedlings per seed[a]			
(denom. df, 195 (2004/2005), 182 (2005/2006), 178 (2006/2007))			
Site	13.7***	14.5***	36.7***
Population	16.5***	0.4	2.2
Site × population	13.7***	0.8	13.7***
Survival to fruiting[b]			
(denom. df, 133 (2004/2005), 165 (2005/2006), 175 (2006/2007))			
Site	17.0***	6.3*	32.6***
Population	18.6***	0.3	0.1
Site × population	25.0***	72.6***	2.5
No. of fruits per plant[a]			
(denom. df, 34 (2004/2005), 105 (2005/2006), 77 (2006/2007))			
Site	96.7***	130.2***	31.3***
Population	11.8**	37.3***	4.9*
Site × population	17.0***	36.3***	9.8**
No. of fruits per seed planted[c]			
(denom. df, 182 (2005/2006), 178 (2006/2007))			
Site	n/a	71.8***	0.6
Population	n/a	40.9***	4.9*
Site × population	n/a	95.7***	4.0*

F-ratios from two-way ANOVA are given. Numerator df = 1 in all cases; denominator df for each of the three experiments is given in brackets after each variable name.
[a]Square-root-transformed before analysis.
[b]Arcsine-square-root-transformed before analysis.
[c]Square-root-transformed except in the 2005/2006 experiment, where the analysis was conducted on $\log_e$(number of fruits per seed planted + 0.1).
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 2 Effects of site (Castelnuovo di Porto, Italy vs Rödåsen, Sweden) and population of origin (Castelnuovo di Porto, Italy vs Rödåsen, Sweden) on *Arabidopsis thaliana* number of fruits per survivor, number of fruits per seedling planted, and flowering start in reciprocal transplant experiments conducted in two successive years (2009/2010 and 2010/2011)

Source of variation	2009/2010	2010/2011
No. of fruits per plant[a]		
(denom. df, 533 (2009/2010), 408 (2010/2011))		
Site	63.8***	193.0***
Population	198.8***	52.4***
Site × population	258.9***	34.9***
No. of fruits per seedling planted[a]		
(denom. df, 603 (2009/2010), 708 (2010/2011))		
Site	104.0***	24.3***
Population	20.6***	55.4***
Site × population	321.0***	77.7***
Flowering start (day of the year)		
(denom. df, 530 (2009/2010), 418 (2010/2011))		
Site	20 988***	4373***
Population	1526***	857***
Site × population	1026***	659***

F-ratios from two-way ANOVA are given. Numerator df = 1 in all cases; denominator df for each of the two experiments is given in brackets after each variable name.
[a]Square-root-transformed before analysis.
*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

recorded (Fig. 2). At the Italian site, the fitness of the Italian genotype was higher than that of the Swedish genotype in all five years (4.9-fold in 2004/2005, $P = 0.004$; 22.2-fold in 2005/2006, $P < 0.001$; 7.7-fold in 2006/2007, $P = 0.005$; 3.1-fold in 2009/2010, $P < 0.001$; and 13.8-fold in 2010/2011, $P < 0.001$). At the Swedish site, the Swedish genotype outperformed the Italian genotype in three of five years (3.6-fold in 2004/2005, $P = 0.016$; 3.5-fold in 2005/2006, $P = 0.022$; and
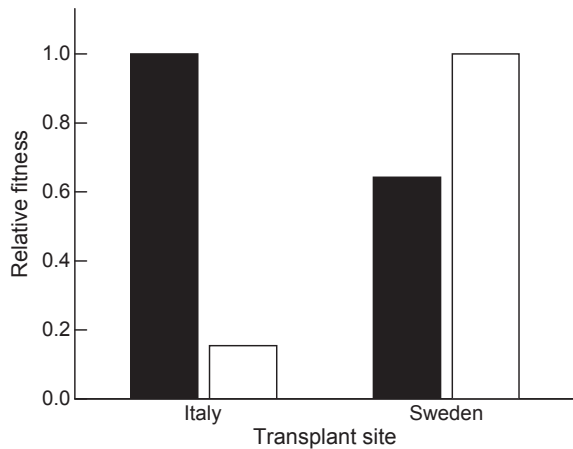
Fig. 3 Mean relative fitness of the Italian (closed bars) and the Swedish (open bars) genotypes of *Arabidopsis thaliana* based on seedling survival and fecundity in reciprocal transplant experiments conducted in five years. Means were weighted by mean fruit production per seedling for each site and year combination.

1.7-fold in 2009/2010, $P < 0.001$); the two genotypes did not differ in fruit production per seed planted in 2006/2007 ($P = 0.86$), or in fruit production per seedling planted in 2010/2011 ($P = 0.33$).

Across the five years, the weighted mean relative fitnesses based on survival and fecundity was markedly higher for the local

genotype than for the nonlocal genotype at both sites (6.7-fold higher in Italy, and 1.6-fold higher in Sweden; Fig. 3).

Fitness components

Differences in survival and in fecundity of surviving plants contributed to the higher overall fitness of the local genotype. At both sites, the local genotype had higher survival than the nonlocal genotype in all five years, and this difference was statistically significant in three of five years at each of the two sites (Figs 4d–f, 5a,b). At the Italian site, reproductive plants of the Italian genotype produced more fruits than the Swedish genotype did in all five years, while at the Swedish site no statistically significant differences in fecundity of reproductives were recorded (Figs 4g–i, 5c,d; Tables 1, 2). Except for the Italian site in the third year, somewhat more seedlings became established in plots planted with Italian seeds than in plots planted with Swedish seeds at both sites; this difference was statistically significant at the Swedish site in the first and third years (Fig. 4a–c; Table 2).

The local genotype produced more seeds per fruit compared with the nonlocal genotype (significant site × population interaction in two-way ANOVA conducted on plot means in the 2005/2006 experiment, $F_{1,92} = 8.1$, $P = 0.005$; 2010/2011 experiment, $F_{1,298} = 74.2$, $P < 0.0001$). Contrasts indicated statistically significant differences in seed number per fruit at one of the sites in the 2005/2006 experiment, and at both sites in the 2010/2011 experiment. In the former experiment, the local
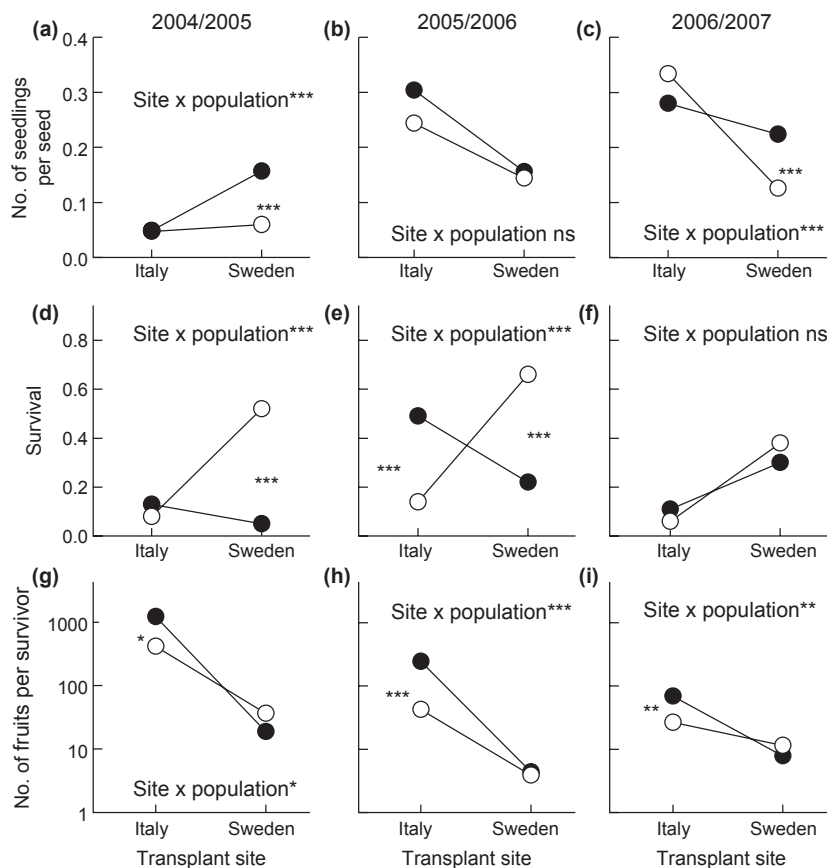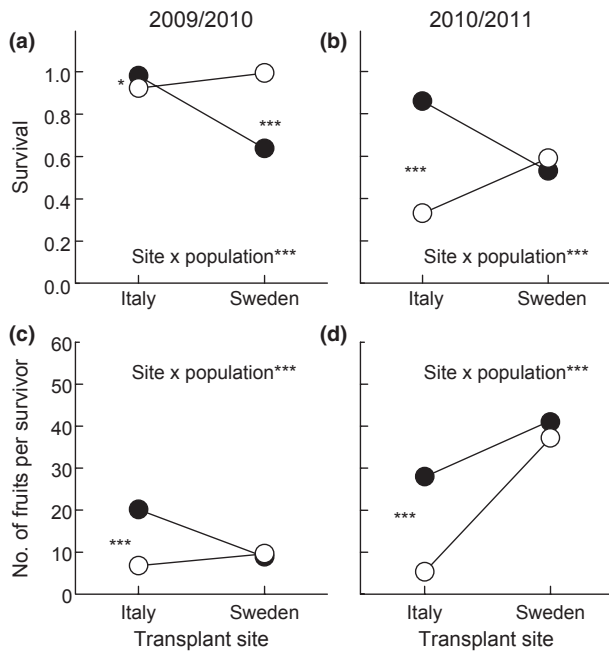

Fig. 4 Seedling establishment, survival and fecundity of the Italian (closed circles) and the Swedish (open circles) genotypes of *Arabidopsis thaliana* in reciprocal transplant experiments in which seeds were planted at the study sites. Indicated are the results of two-way ANOVA conducted on plot means, and of contrasts testing the effect of population separately by site when the site × population interaction was statistically significant. ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Fig. 5 Survival and fecundity of the Italian (closed circles) and the Swedish (open circles) genotypes of *Arabidopsis thaliana* in reciprocal transplant experiments in which seedlings were planted at the study sites. Indicated are the results of nominal logistic regression (survival) and two-way ANOVA (fecundity) examining the effect of site and population separately by year; logistic regression and contrasts were used to test the effect of population separately by site. *, $P < 0.05$; ***, $P < 0.001$.

Fig. 6 Survival of the Italian genotype of *Arabidopsis thaliana* relative to that of the Swedish genotype at the study site in Sweden in relation to the minimum soil winter temperature recorded. Black symbols, years when seeds were planted; gray symbols, years when seedlings were planted at the two sites.

Fig. 7 Flowering start of the Italian (closed circles) and Swedish (open circles) genotypes of *Arabidopsis thaliana* in reciprocal transplant experiments in which seedlings were planted at the study sites. Indicated are the results of two-way ANOVA examining the effect of site and population separately by year; contrasts were used to test the effect of population separately by site. **, $P < 0.01$; ***, $P < 0.001$.

genotype produced 16% more seeds per fruit at the Italian site (mean ± SE based on plot means, Italian genotype 54.3 ± 1.3 seeds, Swedish genotype 46.6 ± 3.1 seeds, $t = 3.2$, $P = 0.002$), while at the Swedish site the difference was not statistically significant (Italian genotype 15.3 ± 1.1 seeds, Swedish genotype 16.8 ± 0.8 seeds, $t = 0.7$, $P = 0.49$). In the 2010/2011 experiment, the local genotype produced 50% more seeds per fruit at the Italian site (mean ± SE: Italian genotype, 36.1 ± 1.3; Swedish genotype, 24.0 ± 1.5, $t = 6.2$, $P < 0.0001$), and 27% more seeds at the Swedish site (Italian genotype, 28.8 ± 0.65; Swedish genotype, 36.6 ± 0.60, $t = 6.4$, $P < 0.0001$). Estimates of correlations between mean number of fruits produced per plant and mean number of seeds per fruit calculated separately by site and genotype were all positive (range: 2005/2006 experiment, 0.22–0.71, $n = 11–38$; 2010/2011 experiment, 0.32–0.79, $n = 23–102$) and statistically significant in three of four cases in the former and in all four cases in the latter experiment.

Relative survival vs winter temperature

At the Swedish site, the relative survival of the Italian genotype increased significantly with the minimum soil temperature recorded (regression coefficient, $b = 0.177$, $n = 5$, $R^2 = 0.97$, $P = 0.012$; Fig. 6), while at the Italian site, where the soil temperature never dropped below 0°C, the relative survival of the Swedish genotype was not associated with the minimum soil temperature ($b = 0.047$, $R^2 = 0.11$, $P = 0.59$).
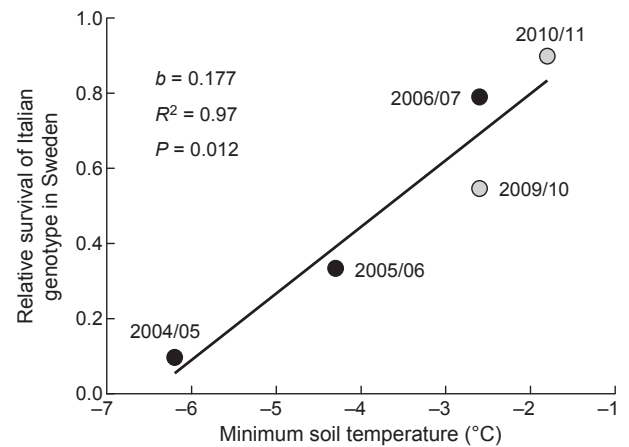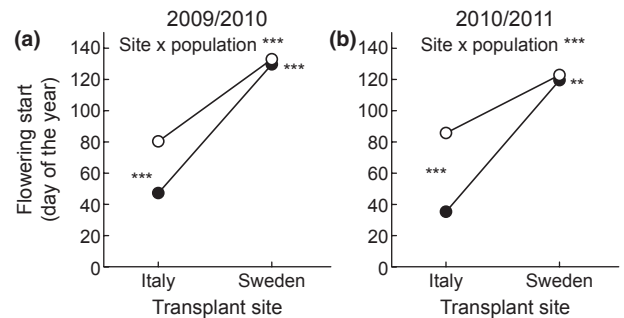
Flowering time

In the experiments initiated with seedlings, the Italian genotype began flowering earlier than the Swedish genotype at both sites, but the difference in flowering time was markedly larger at the Italian than at the Swedish site (significant site × population interaction in two-way ANOVA in both years; Table 2). At the Italian site, the differences in mean flowering time were 33 d (2009/2010 experiment) and 50 d (2010/2011 experiment), while at the Swedish site the differences were 3 d in both years (Fig. 7). In Italy, the Italian genotype began flowering 11 d earlier and the Swedish genotype 6 d later in the 2010/2011 experiment than the year before, while in Sweden both genotypes began flowering 9 d earlier in the final year (Fig. 7). Although we did not collect detailed data on flowering phenology in the experiments initiated with seeds, the same general patterns of early flowering by the Italian genotype and of larger differences in flowering time in Italy than in Sweden were also observed in these experiments.

Discussion

This study has provided evidence of strong adaptive differentiation between two native populations of *A. thaliana*. The local genotype had higher survival than the nonlocal genotype at both sites. At the Italian site, the local genotype also had higher fecundity than the nonlocal genotype. To our knowledge, this is the first demonstration that native *Arabidopsis* populations are reciprocally adapted to their local environments, and also the first example of reciprocal adaptation for any model organism studied in its native environment. The results are consistent with previous documentation of geographic adaptation among populations of other plant species with a wide geographic distribution (Leimu & Fischer, 2008), and suggest that the populations of *Arabidopsis* we studied are highly suitable for further studies of the functional and genetic basis of adaptive differentiation.

At both sites, the local genotype consistently had higher survival than the nonlocal genotype, but the magnitude of this advantage varied among years. At the Swedish site, the relative survival of the Italian genotype was positively related to the minimum temperature recorded in winter. This is consistent with results from laboratory investigations conducted at both the whole-plant and leaf levels, indicating that Swedish genotypes are more resistant to freezing temperatures than Italian genotypes (D. Schemske & J. Ågren, unpublished), and with the general trend of increasing tolerance to cold with increasing latitude of origin in *A. thaliana* accessions (Hannah *et al.*, 2006; Zhen & Ungerer, 2008). At the Italian site, the soil temperature never dropped below freezing during the five years of experiments, and the relative survival of the two genotypes was not related to the minimum temperature in winter. The results suggest that freezing tolerance is a major adaptive trait in Sweden.

The local genotype at the Italian site had higher fecundity than the Swedish genotype, while at the Swedish site no significant differences in fecundity were recorded. The higher fecundity of the Italian genotype at its home site may be related to its ability to grow and initiate flowering under the day length and temperature conditions of the Mediterranean winter. The difference in flowering time may reflect a greater opportunity to escape drought through early flowering at the Italian site because of its long frost-free period before the onset of summer drought. At both sites, the Italian genotype began to flower earlier than the Swedish genotype, but the difference was markedly larger at the Italian site than at the Swedish site (33 vs 3 d in 2009/2010, 50 vs 3 d in 2010/2011; Fig. 7), demonstrating a significant G × E interaction for flowering time. Although G × E interaction for flowering time has been documented in growth room experiments with *Arabidopsis* (Li *et al.*, 2010), this is the first study to quantify its magnitude for plants grown in natural habitats at a time corresponding to the phenology of the local populations. The difference in flowering time between the two genotypes is consistent with evidence for latitudinal clines in flowering time (Caicedo *et al.*, 2004; Stinchcombe *et al.*, 2004), and the earlier initiation of flowering is likely to have contributed to the higher fecundity of the local genotype in Italy.

The magnitude of the home advantage of the local genotype varied among years and was not consistently higher in the three years when seeds were planted than in the two years when seedlings were transplanted. This reflects the fact that most of the local advantage was expressed as differential survival and fecundity, that is, after initial plant establishment. In fact, in two of the three years, the number of seedlings established per seed planted at the Swedish site was higher for the Italian genotype than for the Swedish genotype. In the first year this difference partly balanced, and in the third year, it fully balanced differences in survival and fecundity favoring the Swedish genotype at the northern site.

In this experiment, we used fruit production as an estimate of plant fitness. This may result in inflated estimates of local adaptation if there is a negative correlation between number of fruits produced and number of seeds per fruit. However, our records of seed production instead indicated that the local genotype produced more seeds than did the nonlocal genotype at both the Italian and Swedish sites (16 and 50% more seeds per fruit in Italy, and 10 and 27% more seeds per fruit in Sweden, in the two years examined). The results thus suggest that, if anything, differences in fruit production are likely to underestimate the advantage of the local genotype.

Two previous studies have employed reciprocal transplants to examine local adaptation in *Arabidopsis*. Callahan & Pigliucci (2002) conducted a reciprocal transplant experiment between two populations with differing light regimes located in close proximity in the introduced range (Tennessee, USA). They found no evidence of local adaptation. Arany *et al.* (2008) performed a reciprocal transplant between dune and inland sites in the Netherlands, where the dune sites are native environments historically occupied by *Arabidopsis*, while the inland sites are disturbed roadsides where *Arabidopsis* is a recent colonist. They found that dune plants outperformed inland plants at an experimental garden located near dune populations, while there was no difference in performance between dune and inland populations in the 'inland' garden, possibly indicating insufficient time for inland populations to achieve a local, evolutionary equilibrium. By contrast, the present study demonstrated strong reciprocal adaptation to the local environment of two widely separated populations growing under very different climatic conditions. Taken together, the results are consistent with the expectation that the likelihood of detecting adaptive differentiation should depend on the spatial scale and the steepness of the environmental gradient considered, but also on the time since colonization of a novel habitat.

The present study has identified flowering phenology and freezing tolerance as putative adaptive traits that contribute to the geographic differentiation of *Arabidopsis* populations. In addition to their role in adaptive differentiation along climatic gradients, these traits are also important for understanding evolutionary responses to climate change (e.g. Etterson & Shaw, 2001; Franks *et al.*, 2007). We are currently taking advantage of the genomic tools available for *Arabidopsis* to explore the functional and genetic basis of adaptation.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Air and soil temperature at the two sites in the years of study.

Table S1 Soil minimum temperature (°C) and the number of d with a temperature lower than specified values at the two study sites (Castelnuovo, Italy, and Rödåsen, Sweden)

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