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LOCAL ADAPTATION, PHENOTYPIC DIFFERENTIATION, AND HYBRID FITNESS IN DIVERGED NATURAL POPULATIONS OF *ARABIDOPSIS LYRATA*

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Selection for local adaptation results in genetic differentiation in ecologically important traits. In a perennial, outcrossing model plant *Arabidopsis lyrata*, several differentiated phenotypic traits contribute to local adaptation, as demonstrated by fitness advantage of the local population at each site in reciprocal transplant experiments. Here we compared fitness components, hierarchical total fitness and differentiation in putatively ecologically important traits of plants from two diverged parental populations from different continents in the native climate conditions of the populations in Norway and in North Carolina (NC, U.S.A.). Survival and number of fruits per inflorescence indicated local advantage at both sites and aster life-history models provided additional evidence for local adaptation also at the level of hierarchical total fitness. Populations were also differentiated in flowering start date and floral display. We also included reciprocal experimental F_1 and F_2 hybrids to examine the genetic basis of adaptation. Surprisingly, the F_2 hybrids showed heterosis at the study site in Norway, likely because of a combination of beneficial dominance effects from different traits. At the NC site, hybrid fitness was mostly intermediate relative to the parental populations. Local cytoplasmic origin was associated with higher fitness, indicating that cytoplasmic genomes also may contribute to the evolution of local adaptation.

KEY WORDS: **Brassicaceae, experimental hybrids, fitness, life history, local adaptation, reciprocal transplant experiment.**

Local adaptation arises through spatially varying selection and can lead to genetic and phenotypic differentiation of traits that are targets of the differential selection (Hedrick 2006). Traditional reciprocal transplant experiments in plants have frequently shown evidence for local adaptation, defined as higher fitness of the local population than the nonlocal population at all study sites (e.g., Turesson 1922; Clausen et al. 1948; Kawecki and Ebert 2004; Leimu and Fischer 2008; Hereford 2009). However, studies examining local adaptation in diverged populations inhabiting different continents and climates are currently lacking. In these cases, in addition to relatively rapid adaptive evolution from standing genetic variation (Hermisson and Pennings 2005; Barrett and Schluter 2008; Schluter and Conte 2009), adaptive benefit from new mutations may also underlie the genetic basis of local adaptation. Also, in the absence of gene flow, the fitness effects of the new mutations have not been tested by selection in environmental conditions of other allopatric populations. Thus, fitness effects may be difficult to predict in novel conditions, such as with invasive species (Barrett et al. 2008).

Large differences in environmental conditions and considerable divergence between populations may yield greater phenotypic differences, possibly involving also evolution of life-history-related traits such as longevity, age, and size at time of reproduction, allocation of resources into number of offspring, and sexual and asexual reproduction (Kawecki and Ebert 2004). In plants, local adaptation often involves differentiation in flowering-related traits (e.g., Olsson and Ågren 2002; Hall and Willis 2006; Lowry et al. 2008a; Samis et al. 2008). Flowering should occur when environmental conditions (e.g., temperature) are favorable and seeds should be mature before unfavorable periods, such as seasonal drought or cold temperatures. Cues for these processes are temperature and day length (Chouard 1960; Thomas and Vince-Prue 1996; Simpson and Dean 2002). Plants locally adapted to different latitudes may then be expected to have different responses to photoperiod and other environmental cues. Timing of flowering also needs to coincide with pollinator availability and flowering of potential mates. In addition to timing of flowering, traits involved in floral display (pollinator attraction) can also be under selection especially in outcrossing and self-incompatible plants, depending on pollinator availability (e.g., Sandring et al. 2007). Examining differences in timing of flowering and floral display traits in common garden conditions provides information about aspects of phenotypic differentiation that have a genetic basis.

Adaptive differentiation is caused by selection on traits with potentially different genetic architectures (Mackay 2001; Buckler et al. 2009). The genetic basis of local adaptation and ecologically important traits, and the degree of population differentiation can be examined by reciprocally transplanting experimental hybrids in the habitats of the parental populations or species, and comparing phenotypes of hybrids and parental populations. Accounting for the contribution of different components of fitness in these phenotypic studies can yield important insights into the genetic basis of fitness-related traits (e.g., dominance and epistasis). The F_1 generations often have relatively high fitness because of heterosis, but later-generation hybrids may perform more poorly due to hybrid breakdown (Lynch and Walsh 1998; Lowry et al. 2008b). F_2 hybrid fitness is expected to be low especially when specific epistatic interactions are important for adaptation (Barton 2001), but beneficial effects of hybridization may still be seen in some instances (Rieseberg et al. 1996; Erickson and Fenster 2006). Novel combinations of traits are possible as a result of recombination, as well as transgressive segregation giving rise to a greater range of character values (Rieseberg et al. 1999; Lexer et al. 2003; Lai et al. 2005). Later-generation hybrids can also show hybrid vigor if new combinations of traits or alleles have beneficial effects on fitness (reviewed by Burke and Arnold 2001).

In addition to selection in nuclear genes, cytoplasmic genomes also become genetically diverged, and may contribute to local adaptation (e.g., Galloway and Fenster 2001; Campbell et al. 2008; Sambatti et al. 2008). Cytoplasmic evolution can give rise to phenotypic differentiation (e.g., in flower size), and may even cause incompatibilities between the cytoplasmic and nuclear genomes of different populations through lowered fecundity and viability in hybrids, as has been demonstrated in several plant species (reviewed by Levin 2003).

In this study, we investigated population differentiation and its genetic basis using an outcrossing herbaceous perennial plant *Arabidopsis lyrata* (L.) O'Kane & Al-Shehbaz (Brassicaceae) as our study organism. *A. lyrata* is becoming a model organism for studies of ecological genetics, partly because of comparisons to its close relative *Arabidopsis thaliana* (Koch et al. 2000; Mitchell-Olds 2001; Kuittinen et al. 2004; Clauss and Koch 2006; Koch and Matschinger 2007). Genetic differentiation of *A. lyrata* populations has been extensively studied (e.g., Jonsell et al. 1995; Clauss and Mitchell-Olds 2006; Muller et al. 2008; Ross-Ibarra et al. 2008; reviewed by Savolainen and Kuittinen 2010). Phenotypic differentiation in many potentially adaptive traits such as flowering time and trichome variation have been documented (Riihimäki and Savolainen 2004; Riihimäki et al. 2005; Kivimäki et al. 2007). Local adaptation among European populations has been demonstrated with reciprocal transplant experiments (Leinonen et al. 2009), as well as evidence for selection in the wild on flowering time and floral display traits (Sandring et al. 2007; Sandring and Ågren 2009).

This species has disjunct populations inhabiting low-competition habitats in a variety of climatic conditions around Eurasia and North America (Jalas and Suominen 1994; O'Kane and Al-Shehbaz 1997). European and North American populations are defined as separate subspecies: *A. lyrata* ssp. *petraea* in Europe and ssp. *lyrata* in North America (Al-Shehbaz and O'Kane 2002). The range of *A. lyrata* has expanded from refugia since the last glacial maximum, presumably from central Europe (Koch and Matschinger 2007; Ross-Ibarra et al. 2008; Schmickl et al. 2010) and, separately, across into North America. Limited opportunities for gene flow, combined with the potential for divergent selection on preexisting genetic variation and acquisition of new mutations favorable in the new environments, make populations of *A. lyrata* ideal natural laboratories to gain insights on the degree to which plant species can evolve adaptations to different climates and the genetic nature of these adaptations.

We examined the extent of adaptive differentiation and contribution of individual fitness components to adaptation in allopatric and effectively isolated natural populations of *A. lyrata* from different environments (Norway and NC) using reciprocal transplant experiments of parental populations and their experimental hybrids. A key feature of our experimental approach is that we evaluate both overall fitness, using biologically and statistically appropriate hierarchical models, and individual fitness

components within these hierarchies to understand how differences in individual components contribute to overall fitness differences. We ask the following questions: first, is there evidence for local adaptation at the level of overall fitness in these populations? Second, how do individual fitness components contribute to overall fitness differences of the populations in their respective native environments? Third, has adaptation to different environmental conditions yielded differences in putatively adaptive traits (flowering time and floral display)? Fourth, what is the contribution of individual fitness components to overall F_1 and F_2 hybrid fitness, and do hierarchical total fitness or individual fitness components in hybrids at the two sites show evidence of hybrid breakdown or, alternatively, heterosis? Fifth, does cytoplasmic origin contribute to fitness differences, as evidenced by differences between reciprocals within each hybrid class.

Material and Methods

PLANT MATERIAL

Geographically and genetically distant populations representing *A. lyrata* ssp. *petraea* and *A. lyrata* ssp. *lyrata* were chosen for our study. Populations originated from habitats with different environments: from an alpine valley with relatively short growing season in Spiterstulen (*A. lyrata* ssp. *petraea*), Norway in Europe (61° 38′N, 8° 24′E,1106 m.a.s.l.) and from a warm temperate climate in Mayodan (*A. lyrata* ssp. *lyrata*), NC, near the southern range limits of *A. lyrata* in the United States (36°25′ N, 79°58′ W, 225 m.a.s.l.). Habitats of these populations represent climatic extremes in temperature across the range of *A. lyrata* and also differ in photoperiod. Based on data from microsatellite loci, our study populations are highly differentiated (F_{st} = 0.668; Muller et al. 2008). Preliminary analysis of sequence variation between these populations suggests that they diverged quite recently (Pyhäjärvi et al., unpublished), concordant with earlier results on related populations by Ross-Ibarra et al. (2008), who estimated a divergence time of 35,000 years for North American and Central European populations.

Plants grown from seeds collected in the field in Mayodan, NC, (Ma; provided by C. Langley) and in Spiterstulen, Norway (Sp) were grown and crossed in long-day conditions in growth chamber at the University of Oulu to obtain seed material for the field experiments. Within-population crosses were conducted to get unrelated full-sib families representing the parental populations. Three unrelated plants from each population were reciprocally crossed to generate three unrelated interpopulation full-sib F_1 families. Two randomly chosen unrelated F_1 individuals from different families, one with a Ma maternal parent and the other with a Sp maternal parent, from different populations were grown and crossed reciprocally in the growth chamber to obtain F_2 progeny seeds with cytoplasms of different origin. This crossing design allowed us to avoid problems with self-incompatibility. Although some populations in North America have been reported to have lost self-incompatibility (Mable et al. 2005), no such individuals have been found in the populations included in this study.

RECIPROCAL TRANSPLANT EXPERIMENTS

To determine parental and hybrid fitness and differentiation in ecologically important traits in the wild, reciprocal common garden experiments were established in Spiterstulen Norway and in Greensboro, NC, 44 km SE of the site of the Mayodan population. Seeds from the two parental populations, F_1 and F_2 progeny were sown in 2005. Sowing and pre-growing was done in controlled conditions to gain better establishment success. Seeds were sown in early June 2005 in Norway using a mix (1:1) of sand from the local area and commercial planting soil (Hasselman), and in September 2005 in NC using Fafard germinating mix. After sowing in NC, the pots were kept at 4 °C for a week. Pre-growing was done in a greenhouse in Norway (natural day length approximately 19 hours) and in growth chambers in NC at an initial day length of 16 hours. In NC, plants were acclimated gradually for lower temperatures and shorter day lengths prior to planting.

To account for environmental variation within sites, the transplant experiments were conducted in a randomized common garden design. Plants representing different populations and families were randomized in eight blocks at both sites. Randomization was done at the time of sowing in Norway and before planting in NC. The experiment in Norway included 98 plants from the Spiterstulen population (Sp) and 120 plants from the Mayodan, NC (Ma) population represented by 6 and 10 unrelated full-sib families, respectively ("Sp2" n = 11, "Sp4" n = 7, "Sp6" n = 19, "Sp7" n = 17, "Sp8" n = 5, "Sp9" n = 8, "Sp10" n = 6, "Sp13" n = 12, "Sp14" n = 9, "Sp16" n = 4 and "Ma1" n = 20, "Ma2" n = 29, "Ma3" n = 24, "Ma4" n = 8, "Ma9" n = 15, "Ma10" n = 24). The experiment at the NC site included 131 plants from the Sp population and 86 plants from the Ma population, represented by and 6 unrelated full-sib families, respectively 5 ("Sp1" n = 27, "Sp2" n = 28, "Sp4" n = 16, "Sp5" n = 26, "Sp9" n = 21, "Sp10" n = 13 and "Ma1" n = 15, "Ma2" n = 7, "Ma3" n = 20, "Ma9" n = 29, "Ma10" n = 15). Four families from the Sp population and five families from the Ma population were the same at both sites. At the Norway site, a total of 186 F_1 plants and 479 F_2 plants were planted. F_1 plants represented three unrelated families (F_1-a: n = 34, F_1-b n = 35, F_1-c n = 117), and all of them were represented by both reciprocals. Of the hybrid plants in Norway, 77 F_1 and 239 F_2 plants had Sp cytoplasm (named "$SpMaF_1$" and "$SpMaF_2$") and 109 F_1 and 240 F_2 plants had Ma cytoplasm (named "$MaSpF_1$," "$MaSpF_2$"). At the NC site, a total of 397 F_2 plants were included, represented by 226 plants from $SpMaF_2$ reciprocal and 171 plants from $MaSpF_2$. Only nine F_1

plants were available for planting at the NC site because of poor germination. Germination success was in general relatively good for all other seeds at both sites, and there were no observable difficulties in producing the experimental hybrids.

The experiment in Norway was planted in the end of June 2005 and the NC experiment in November 2005, with planting times chosen to maximize the probability of overwinter survival. Individuals were planted with 30 cm spacing in NC, in which larger vegetative size was to be expected and 10 cm in Norway, in which planting space was limited and plants were expected to be relatively small, based on earlier work at the same site (Leinonen et al. 2009).

TRAIT MEASUREMENT

We measured fitness components in central life history stages in both environments to be able to estimate their contribution to total fitness. We define survival, flowering propensity, number of inflorescences, fruits and seeds as separate components of fitness. At both experimental fields, plants were scored as alive or dead in the beginning and in the end of the growing season and as having been flowering or vegetative that year. At the end of the flowering period and fruit ripening, inflorescences and fruits were counted and 1–3 fruits were sampled to estimate seed production as they ripened. At the NC site, inflorescences were counted and the average number of fruits per inflorescence was estimated from a sample of three inflorescences when more than five reproductive inflorescences were present. Six to 12 ripe fruits per plant were sampled to estimate seed production at the NC site. The average number of seeds per fruit was then calculated based on seed number in sampled fruits. Seeds that seemed aborted and clearly nonviable were not included in these counts. At the NC site, a small amount of additional flowering occurred after data were collected, but this would have made a minimal contribution to seasonal reproductive output.

To examine differentiation in putatively adaptive traits, flowering start date and floral display (inflorescence length, petal size, and flower number) were also measured. Flowering start date was determined as the day when the first flower was open. At this stage, the length of the inflorescence with the first flower was measured. In Norway, when a flower was fully open, the length and width of the visible part of one petal from three different flowers were measured with digital calipers. Petal size was calculated using formula of an ellipse. Mean petal size was calculated based on these three measurements, as the sizes in the three different flowers were relatively similar. All flowers that each plant had produced during the first growing season were counted. Flowering start was recorded in three years in Norway, inflorescence length and flower number in the first year and petal size during the first and second year of the study. Data for petal size were combined from the two years.

In Norway, initial measurements in the beginning of the growing season started in late May when snow had melted, and final measurements were done in late August each year (2006–2009). In NC, measurements were started in early March when flowering began, and continued until fruit set was essentially complete in late June. Flowering start was scored every second or third day in Norway and twice per week in NC. Data were collected for four years in Norway, and for a single year at the NC site. At the NC site there was heavy mortality following the reproductive season and a large number of volunteer seedlings germinated in the field plot that fall, the combination of which precluded collecting additional data.

STATISTICAL METHODS

Fitness components were analyzed using generalized linear mixed models (*lmer* function in package *lme4*) in *R* (R Development Core Team 2009). These models treated population as a fixed effect, as the specific populations (Sp, Ma, F_2, and in Norway F_1) were the primary variables of interest. Block and family within population were treated as random effects because they represent sampling of environments within each test location and genetic variation within each population, respectively. Cytoplasmic origin (reciprocal) was used as the family effect for the hybrids, because F_2 were represented by only one actual family.

Some fitness components were square-root transformed to improve the normality of the residuals. For count traits, this approach appeared to be more satisfactory than modeling the data as Poisson (or quasipoisson) distributed, mainly due to a high degree of overdispersion and because some counts were estimated by sampling. Residual normality plots of transformed data, including count data, showed little evidence of skewness or heavy tails. Survival and flowering were modeled as binary variables using a binomial family and logit link in the mixed models. Maximum likelihood was used as the criterion because some of the tests involved comparisons with reduced models that had different fixed effects. The significance of differences between the populations and hybrids at each site were determined using hierarchical likelihood ratio tests, comparing the full model with all population categories with a model in which the studied pair of populations was combined as one population category.

We performed additional analyses to explore differences in total fitness in more detail with aster models for life-history analysis in *R* to account for the hierarchical structure of fitness (Geyer 2007; Shaw et al. 2008). Total fitness was modeled as a hierarchical function of multiple life-history stages: survival, flowering, and reproductive output (Fig. 1). In this way, many components of fitness with different statistical distributions could be included in the same analysis, and reliable statistical tests and error estimates for total fitness could be generated. When life-history stages are estimated by subsampling, appropriate methods for

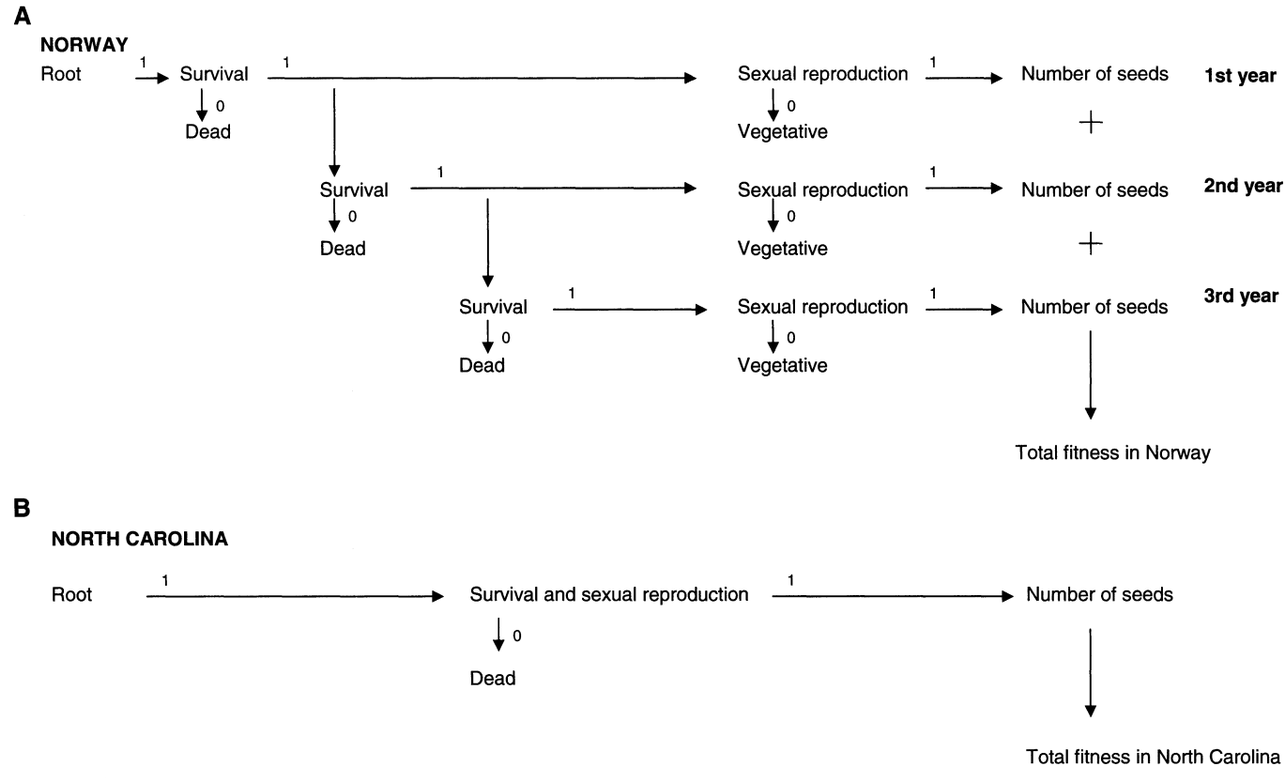

Figure 1. Hierarchical structure of life-history stages used in aster models for life-history analysis for dataset from (A) Norway and (B) NC sites for estimating total fitness. The three sampled count life-history stages (number of inflorescences, fruits per inflorescence and seeds per fruit) were combined into one for total reproductive output conditional on flowering at both sites and two binary life-history stages (survival and flowering conditional on survival) were combined into one binary life-history stage describing whether the original plant flowered for the NC site.

data collection should be used (p44 in Shaw et al. Technical Report 661).

Because the method was published after our data was collected, our sampling method violated the set of conditions under which the total reproductive output or some of its components, conditional on flowering, strictly follow the zero-truncated negative binomial distribution that can be modeled in aster. However, leaving these life-history stages out of the analysis (especially fruits per inflorescence, which had a highly significant difference between populations) would have resulted in biased fitness estimates due to the contrasting patterns of population differences for individual life history stages (see Results). The complete aster hierarchy for total fitness for a single year of data is: root → survival to start of flowering season (binary) → flowering (binary) → number of reproductive inflorescences (count) → fruits per inflorescence (sampled count) → seeds per fruit (sampled count). Because of the issues in sampling, we tried two kinds of aster models and examined which one provided better fit by examining residuals. First, aster models were fitted using zero-truncated negative binomial distribution, with non-integer estimates of the life-history stages rounded to the nearest integer. We obtained maximum-likelihood estimates of size parameters to correctly scale the variance proportional to the mean using a grid search and spline interpolation procedure in *R*. Methods were adapted from pp. 43–45 and 69–70 of Shaw et al., Technical Report No. 658 (http://www.stat.umn.edu/geyer/aster/). Including each stage of the above complete hierarchy in the aster models resulted in information matrices that were generally inestimable due to convergence failures or singularity. Consequently, we combined the two binary life-history stages (survival and flowering conditional on survival) into one binary life-history stage describing whether the original plant flowered for the NC data. For both sites, the three count life-history stages were combined into one for total reproductive output conditional on flowering, which was rounded to the nearest integer. Second, because of the statistical issues with applying the negative binomial model to life history stages that were sample means rather than true counts, we also modeled reproductive output conditional on flowering as a normally-distributed life-history stage in aster after applying appropriate transformations. Square-root transformation for data from the NC site and

cube-root transformations for Norway site resulted in residual plots that were reasonably close to normal distributions when linear models corresponding to the conditional trait distributions were analyzed for flowering plants using the *lm* function in *R*. The residual standard deviations from these analyses were used as input into aster, which requires a known standard deviation when data are modeled with a normal distribution.

Aster models with transformed normal distribution provided a better fit than the zero-truncated negative binomial based on the fit of the residuals. Therefore we chose to use the transformed normal models to examine differences in total fitness in our data. This was done using hierarchical likelihood-ratio tests in which pairs of populations were combined in the reduced models to test for significant pair-wise differences. To test specifically for the advantage of the local population or cytoplasm, we used one-tailed tests. All other comparisons were two-tailed.

To get a robust estimate of the significance of hybrid breakdown in individual fitness components due to epistatic gene action, separate *lmer* models with orthogonal contrasts were used with the Norway data, in which we had suitable sample sizes for F_1 progeny and both parental populations. In the absence of epistasis, the expected mean trait value for the F_2 generation is equal to the average of the parents and F_1 generation, and significant deviations from this expectation provide evidence for epistasis (Whitlock et al. 1995; Fenster et al. 1997; Lynch and Walsh 1998, p. 206–209; Johansen-Morris and Latta 2006). Statistical significance of the contrast of the F_2 versus a 1:2:1 ratio of Sp: F_1:Ma was estimated with 1000 MCMC (Markov Chain Monte Carlo) runs to get confidence intervals using the *HPDinterval* function in *R* for continuous fitness components, and test statistics from the summary table of the model for binary fitness components. Only negative deviations of the F_2 from the expected value are interpreted as hybrid breakdown due to negative epistatic interactions between Sp and Ma alleles (or loss of favorable interactions of intrapopulation combinations). Positive deviations would support favorable epistatic interactions of Sp and Ma alleles, which are not expected, but could also represent maternal effects due to heterosis in the F_1 generation (Frascaroli et al. 2007).

Additional *lmer* models and likelihood-ratio tests were performed to test for cytoplasmic differences between F_1 and F_2 reciprocals in the studied fitness components. Because our F_1 progeny included plants from three unrelated families, both family and reciprocal were included in the model as fixed effects when differences between F_1 reciprocals were tested. Significance was estimated with likelihood-ratio tests between models in which the reciprocal factor was present and removed.

Differentiation in flowering start and floral display traits was analyzed with *lmer* models and tested for dominance, epistasis, and the contribution of cytoplasm in a similar way as fitness components. Flowering time data were clearly nonnormal even after transformation, so a nonparametric Kruskal–Wallis test was also performed to examine differences between populations, using the *kruskal.test* function in the *R* stats package.

We also examined selection patterns in the F_2 plants on flowering start and floral display traits, using regressions of fitness on linear and quadratic combinations of these traits. F_2 hybrids are especially informative for a selection analysis, because allelic combinations from different populations are broken up by recombination, minimizing non-functional correlations of phenotypes with fitness. We chose not to examine trait regressions on fitness among the parental populations, because this would have required an analysis within each family, and this was not possible because of too low sample size per family. We tried using aster models to analyze linear and quadratic effects of phenotypic traits on fitness. However, transformation of fitness as a dependent variable in these analyses can produce incorrect inferences of the nature of selection (Lande and Arnold 1983, Stanton and Thiede 2005, Shaw et al. 2008), precluding the use of a transformed normal distribution of reproductive output conditional on flowering in the fitness hierarchy. When we used the zero-truncated negative binomial distribution instead to model the conditional distribution of reproductive output in aster models, all tested quadratic models for the dataset from both sites as well as linear models from the Norway site either contained zero or negative eigenvalues or failed to converge, and were thus apparently not estimable even when lowering the information threshold as recommended by Shaw et al. (http://www.stat.umn.edu/geyer/aster/). This was likely due to poor fit of the negative binomial model in general for our data. Thus, only estimates of linear selection are reported, and only for the NC dataset. All analyses were performed with *R* versions 2.7.2, 2.9.2, and 2.10.0 (R Development Core Team 2009).

Results

LOCAL ADAPTATION AND FITNESS DIFFERENCES BETWEEN THE PARENTAL POPULATIONS

Evidence for local adaptation (local superiority at both sites) was seen in survival (Figs. 2A, B) and number of fruits per inflorescence (Figs. 4C, D). Other fitness components showed local advantage in only one of the two sites (but in Norway over several years). Statistical significance of differences between the parental populations is shown in Table 1. In Norway, 89% of the local Sp plants had survived after four years (Fig. 2A). By contrast, the nonlocal Ma population experienced drastic mortality beginning with the second winter, and after four years, only 1% of the plants were alive. Plants from both populations had high flowering propensity in the first year (Sp: 89%, Ma: 96%; Fig. 3A). The percentage of the local Sp plants that flowered, however, was

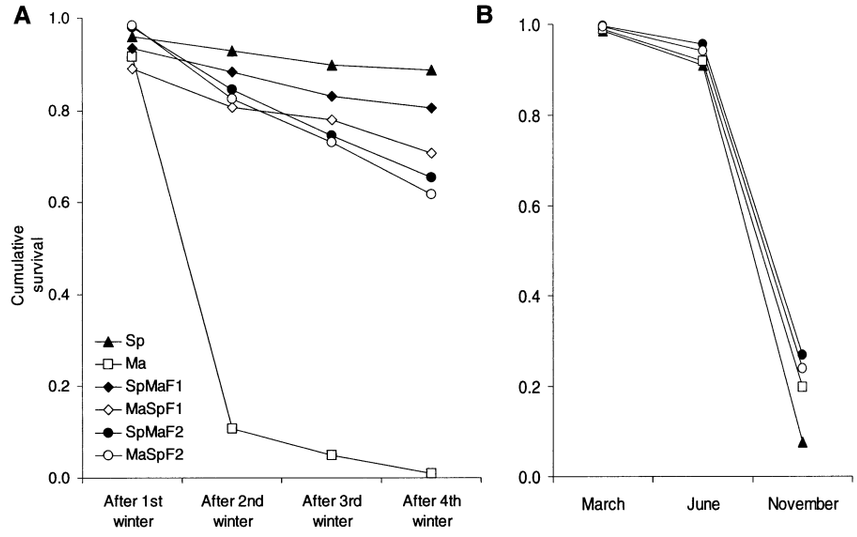

Figure 2. Cumulative survival (proportion of plants alive of those initially planted) of parental populations Spiterstulen Norway (Sp) and Mayodan North Carolina US (Ma) and F1 and F2 hybrid reciprocals over (A) four years in Norway and (B) one year in NC.

only 50% or less in later years, as many Sp plants stayed vegetative. The remaining few nonlocal plants had higher flowering percentages in years 2 and 3, but the difference was not significant. The local Sp population also produced significantly fewer inflorescences on average in the first and second year on plants that flowered than did the nonlocal population (Fig. 4A). In contrast, the Sp plants had significantly more fruits per inflorescence and seeds per fruit than the nonlocal population in the first and second year (Figs. 4C, E).

At the NC site, more than 90% of plants from both populations survived over the winter and their first reproductive season until June, but heavy mortality for both populations occurred later that year (Fig. 2B). Nearly all local Ma plants flowered, but ~60% of the nonlocal plants stayed vegetative (Fig. 3B). Plants from the Ma population that flowered produced significantly more inflorescences (Fig. 4B), and fruits per inflorescence compared with the nonlocal plants (Fig. 4D). Seed number per fruit did not differ significantly between the populations at this site (Fig. 4F). In general, values for all three components of seed output were higher for both populations planted in NC than for any one year in Norway.

Aster analysis of hierarchical total fitness also showed evidence for local adaptation (fitness advantage of the local population at both sites) between the study populations (Table 2). In Norway, hierarchical total fitness of the local Sp plants over three years was 35% greater than that of the nonlocal population (Fig. 5A), and the difference was significant (Table 2). At the NC site, plants from the local Ma population had more than a hundredfold advantage in total fitness in one season (Fig. 5B), and the difference was highly significant according to aster analysis (Table 2).

ECOLOGICAL DIFFERENTIATION

Both flowering start date and floral display traits showed genetically-based differences between populations, indicating that the differentiation could be a result of different selection pressures. Indeed, the selection analysis with nested likelihood ratio tests of the F_2 plants with linear models in NC showed evidence for selection for early flowering (linear selection coefficient $\beta = -2.23 \times 10^{-5}$, d.f. = 1, deviance = 10.3, $P = 0.0013$) and long inflorescences ($\beta = 2.23 \times 10^{-6}$, d.f. = 1, deviance = 38.5, $P < 0.0001$), corresponding to the phenotype of the local population.

The local population flowered significantly earlier than the nonlocal population at both sites in the first year (Table 3). Sp plants opened their first flower eight days earlier on average than Ma plants in Norway in the first year (Fig. 6A). In contrast, in NC the Ma plants were early-flowering, with a mean difference of 16 days between parental populations (Fig. 6B). The local Sp population also flowered earlier in the second and third year in Norway, but the difference was significant only for the second year with the nonparametric test (Table 3). Plants from the Sp population had shorter inflorescences at both sites (Figs. 7A, B), and at the Norway site, also had significantly smaller petals (Fig. 7C) and significantly fewer flowers (Fig. 7D) on average than the plants from the Ma population (Table 3).

HYBRID PHENOTYPES AND THE GENETIC BASIS OF FITNESS AND ECOLOGICAL DIFFERENTIATION

Hybrid phenotypes were used in multiple ways in our analyses. First, we examined what kinds of gene action fitness components and traits describing ecological differentiation show. Second, at each site, we studied deviations of F_1 and F_2 hybrid performance

Table 1. Local adaptation and gene action for fitness components. X^2 values and significance of likelihood ratio tests of *lmer* models (d.f.=1) are shown for the sites in Norway and NC.

Trait	Year	Nor: Sp-Ma		Nor: Sp- F1		Nor: Ma- F1		Nor: Sp- F2		Nor: Ma- F2		NC: Ma-Sp		NC: Ma- F2		NC: Sp- F2	
		X^2	P	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P	X^2	P
Surv.	4 or 1	55.01	<0.0001	8.41	0.0037	35.67	<0.0001	15.78	0.0001	33.36	<0.0001	6.94	0.0084	1.48	0.2233	9.49	0.0021
Fl.%	1st	3.97	0.046	5.59	0.018	0.11	0.7452	8.24	0.0041	1.09	0.2957	24.2	<0.0001	1.25	0.2641	13.94	0.0002
	2nd	1.44	0.23	17.69	<0.0001	3.66	0.0556	16.46	<0.0001	2.72	0.0994						
	3rd	1.64	0.2	18.03	<0.0001	1.89	0.1695	13.79	0.0002	0.6	0.4399						
	4th	1.12	0.29	12.23	0.0005	2.65	0.1033	8.49	0.0036	2.03	0.1544						
#infl.[1]	1st	29.19	<0.0001	17.45	<0.0001	3.49	0.0619	19.2	<0.0001	2.24	0.1343	44.3	<0.0001	12.65	0.0004	31.41	<0.0001
	2nd	7.83	0.005	7.32	0.0068	2.67	0.1023	7.86	0.0051	2.9	0.0887						
	3rd	0.79	0.375	6.88	0.0087	0	0.9698	8.7	0.0032	0.01	0.9276						
#fr./infl.[1]	1st	30.34	<0.0001	8.02	0.0046	15.2	0.0001	7.24	0.0071	16.51	<0.0001	37.7	<0.0001	22.51	<0.0001	11.94	0.0006
	2nd	10.63	0.001	2.00	0.1577	7.76	0.0054	8.66	0.0033	4.6	0.032						
	3rd	0.41	0.52	10.18	0.0014	0.89	0.3449	7.72	0.0055	0.31	0.5778						
# seeds/fr.[1]	1st	29.82	<0.0001	26.56	<0.0001	0.85	0.3571	28.96	<0.0001	3.61	0.0573	2.39	0.1221	4.46	0.0346	1.08	0.2984
	2nd	15.73	<0.0001	8.84	0.003	6.48	0.0109	10.05	0.0015	4.87	0.0274						
	3rd			4.15	0.0417			4.31	0.0379								

[1]Square-root transformation.

from expected values, indicating hybrid breakdown—or alternatively shows hybrid vigor (heterosis) due to dominance or favorable epistatic interactions—at the level of hierarchical total fitness. Third, we searched for evidence of cytoplasmic effects on the studied fitness components and traits.

Gene action for fitness components

Some components of fitness showed that the hybrid phenotype was intermediate and significantly different from both higher and lower parent and in some cases the hybrids differed significantly from only one of the parents (providing evidence for dominance)(Sp- F_1 and F_2 and Ma- F_1 and F_2 comparisons in Table 1). Deviations of F_2 mean trait values from the average of midparent and F_1 values were not significant for fitness components, providing no evidence for hybrid breakdown or other epistatic effects (Table 4).

Flowering propensity, number of inflorescences, and number of fruits per inflorescence in the third year showed dominance in Norway, as did survival and flowering propensity in NC, and the hybrids resembled the higher (Ma) parent (Table 1). Furthermore, survival of the hybrids in Norway was relatively high, and was closer to the higher (Sp) parent, especially after second and third winter (Fig. 2A). Flowering propensity of F_1 plants in Norway was nearly significant for overdominance in the second year ($P = 0.056$; Table 1). Hybrids resembled the lower parent in number of seeds per fruit both in Norway and in NC in the first year (Ma in Norway and Sp in NC; Table 1). The F_2 had slightly less seeds per fruit in Norway than the nonlocal parent ($P = 0.057$; Table 1). Intermediate performance of the hybrids compared with the parent was seen in Norway in survival after four years, number of fruits per inflorescence and seed number per fruit in the first year, and at the NC site in number of inflorescences and number of fruits per inflorescence (Table 1).

Gene action for flowering start date and floral display

Flowering start date, inflorescence length and petal size in the first year of the F_2 were intermediate in Norway compared to the parental populations. The F_1 plants had an equal number of flowers, and the F_2 even more than the parent with higher flower number (Ma; Table 1; Fig. 7D). Flower number of the F_2 plants indicated significant positive epistasis (Table 4). In NC, the mean F_2 flowering start date resembled the late-flowering Sp population (Table 3).

Contrasting patterns of hybrid fitness across sites

We found that dominance of the better-performing parent for flowering propensity (Fig. 3A) and number of inflorescences (Fig. 4A), and partial dominance for survival (Fig. 2A) contributed to the observed hybrid vigor at the Norway site. There was no clear evidence for true overdominance, except for flowering propensity in

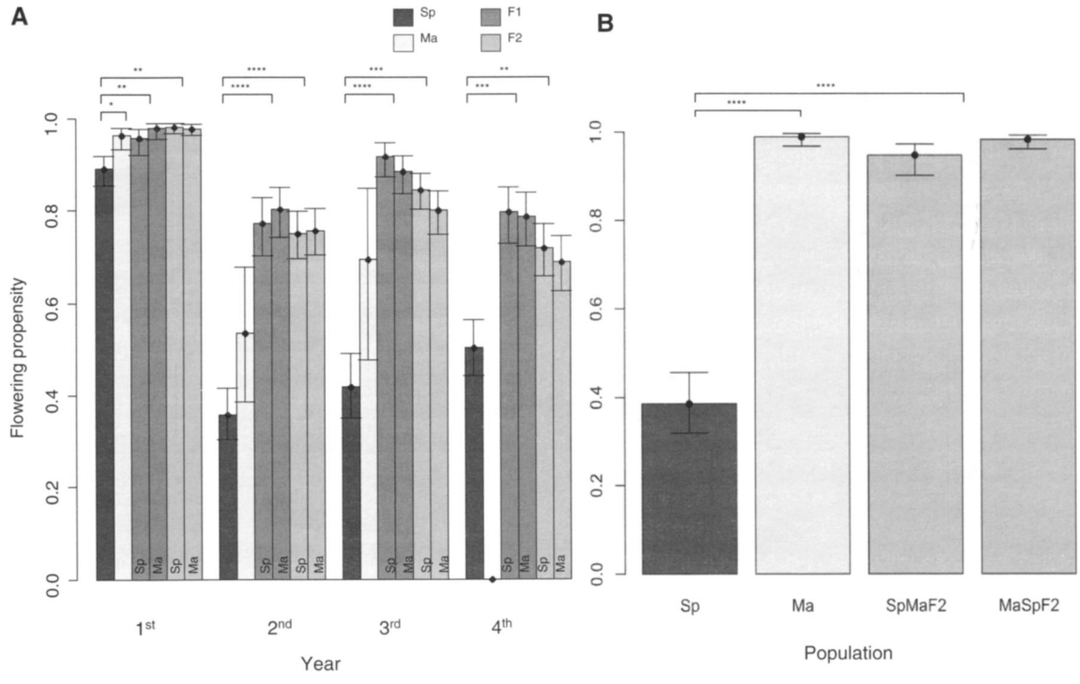

Figure 3. *lmer*-estimates and ± 1 SE for flowering propensity of parental populations Spiterstulen Norway (Sp) and Mayodan North Carolina US (Ma) and F1 and F2 hybrid reciprocals ("Sp" and "Ma" indicate cytoplasmic origin) for (A) four years in Norway and (B) one year in NC. Colors indicate populations at the Norway site (see legend). Significant pair-wise differences are indicated above brackets: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$.

one year in the F_1 generation, nor for either hybrid breakdown or favorable epistatic interactions (Table 1). Only seed number per fruit of the F_2 in the first year was at the level of the lower parent at both sites.

In contrast, at our study site in NC, most fitness components showed intermediate values compared with the parents for F_2, with the exception that survival and flowering propensity showed dominance (Table 1; Figs. 2B and 3B). Fruits per inflorescence and seeds per fruit in the F_2 was significantly lower than the fitness of the best-performing local parent, and closer to the poorer-performing parental population (Figs. 4D, F).

Hybrid breakdown or heterosis?

In comparisons of hierarchical total fitness between the parents and hybrids using aster models, we found that in Norway, hierarchical total fitness of the hybrids significantly exceeded that of the local parent, indicating heterosis at this site (Table 2). F_1 hybrid total fitness was 7% higher and F_2 fitness 9% higher than that of the local population (Fig. 5A). The F_2 plants did not differ significantly from the F_1 in hierarchical total fitness, indicating that fitness did not decrease even in later-generation hybrids. In the NC environment, heterosis was absent, as the local parental Ma plants had a threefold fitness advantage over the F_2 plants (Fig. 5B), and this difference was also highly significant (Table 2). However, the lack of sufficient F_1 data precluded a specific test for hybrid breakdown in NC.

Cytoplasmic effects on local adaptation and ecological differentiation

Local cytoplasm was associated with high fitness in some of the measured components of fitness. At the site in Norway in the third year, the F_1 reciprocal with the local cytoplasm ($SpMaF_1$) had significantly more fruits per inflorescence than the other reciprocal (Table 5; Fig. 4C). In the second year at the same site, the F_1 with the nonlocal cytoplasm ($MaSpF_1$) had more seeds per fruit that the other reciprocal (Table 5; Fig. 4E). In NC, the $MaSpF_2$ (with Ma cytoplasm) had significantly more seeds per fruit, and was nearly significant for higher flowering propensity ($P = 0.056$) and number of inflorescences ($P = 0.061$) than the other reciprocal,(Table 5; Fig. 3B). Other fitness components did not differ significantly between reciprocals.

Hierarchical analysis of total fitness between reciprocals using aster models showed advantage of the local cytoplasm at both sites for the F_2 generation, indicating a cytoplasmic contribution in local adaptation (Table 2). The F_1 and F_2 reciprocals with local cytoplasm had ~20% higher total fitness than the corresponding reciprocals with nonlocal cytoplasm in Norway, (Fig. 5A). In NC, the reciprocal with the local cytoplasm

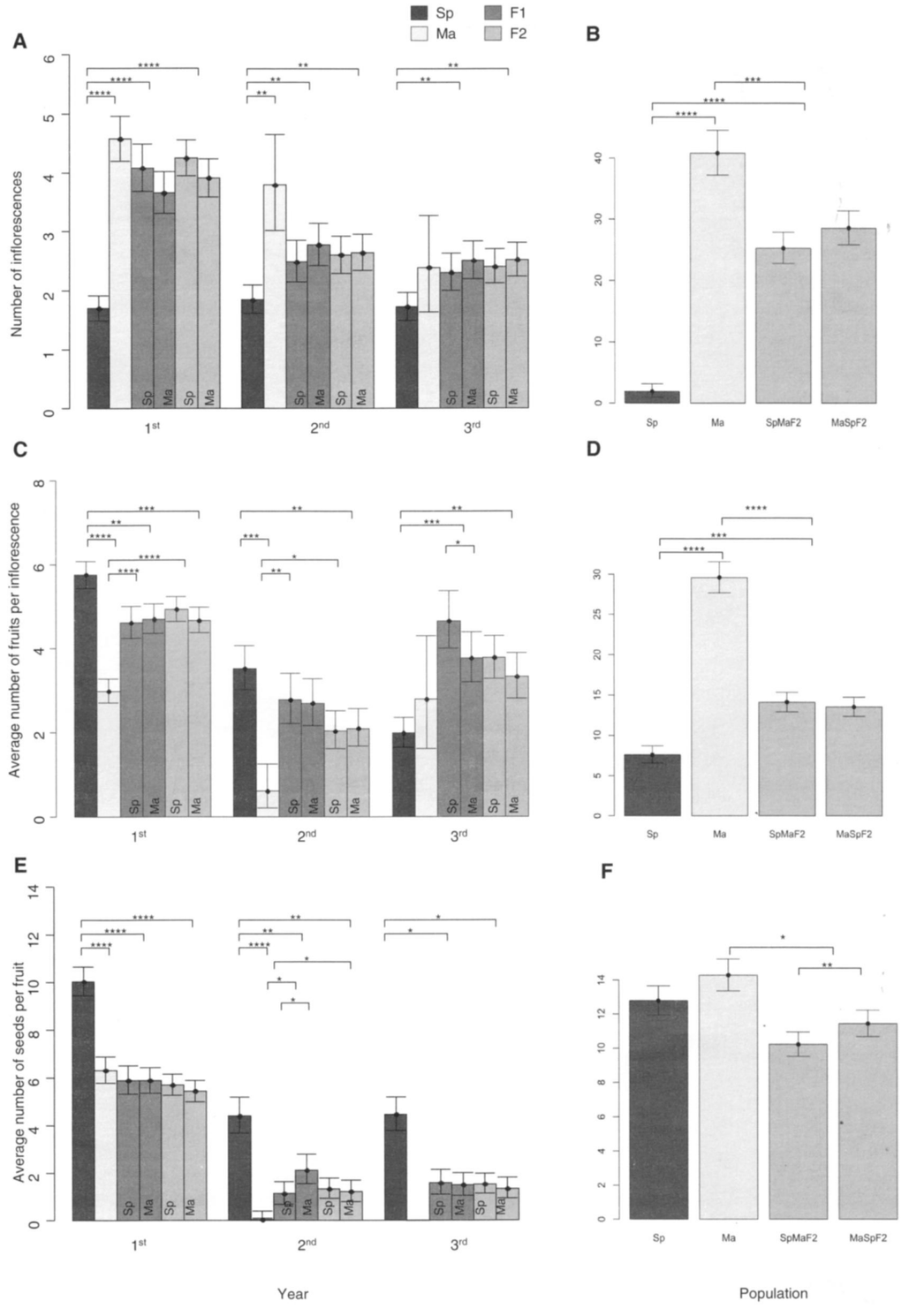

Figure 4. *lmer*-estimates and ± 1 SE for count fitness components: (A, B) number of inflorescences, (C, D) number of fruits per inflorescence, and (E, F) number of seeds per fruit for parental populations Sp Norway and Ma North Carolina US and F1 and F2 hybrid reciprocals ("Sp" and "Ma" indicate cytoplasmic origin) for (A, C, and E) three years in Norway and (B, D, and F) one year in NC. Estimates and SE were inverse-transformed in cases in which the models were fitted on transformed data. Only plants that flowered each year are included. Colors indicate populations at the Norway site (see legend). Note the different scales on the y-axes. Significant pair-wise differences are indicated above brackets: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

Table 2. Local adaptation (Sp vs. Ma), heterosis/hybrid breakdown (local vs. hybrid), and cytoplasmic adaptation (reciprocal comparison) in total fitness. Hypotheses, deviance and *P*-value for likelihood ratio tests (d.f.=1) of aster life history models with transformed normal distribution are shown for the sites in Norway and NC.

Site	Hypotheses			Aster Results	
	H_0	H_1	Explanation	Deviance	*P*
Nor	Sp≤Ma	Sp>Ma	Local adaptation[1]	13.3	0.0001
	Sp=F1	Sp≠F1	Heterosis or incomplete dominance[2]	11.9	0.0006
	Sp=F2	Sp≠F2	Heterosis or incomplete dominance[2]	12.7	0.0004
	F1=F2	F1≠F2	Various	0.2	0.6642
	SpMaF1≤MaSpF1	SpMaF1>MaSpF1	Cytoplasmic adaptation[1]	1.6	0.1042
	SpMaF2≤MaSpF2	SpMaF2>MaSpF2	Cytoplasmic adaptation or nuclear-cytoplasmic interaction[1]	4.1	0.0210
NC	Ma≤Sp	Ma>Sp	Local adaptation[1]	630.8	<1e-10
	Ma=F2	Ma≠F2	Heterosis or incomplete dominance[2]	277.1	<1e-10
	MaSpF2≤SpMaF2	MaSpF2>SpMaF2	Cytoplasmic adaptation or nuclear-cytoplasmic interaction[1]	4.5	0.0300

[1]One-tailed test.
[2]Two-tailed test.

had 35% higher total fitness than the other reciprocal (Table 2; Fig 5B).

Cytoplasmic origin was also found to contribute to traits involved in ecological differentiation (Table 5). There was a significant cytoplasmic effect for flowering start date and inflorescence length at the site in NC (Figs. 6B and 7B) and for petal size at the Norway site (Fig. 7C). The differences corresponded to those observed between the parental populations except for inflorescence length in NC, in which the F_2 reciprocal with Sp cytoplasm had taller inflorescences than those with NC cytoplasm. Flower number at the Norway site also showed a trend of being influenced by the cytoplasmic background, as the F_2 plants with Sp cytoplasm had more flowers ($P = 0.051$) (Table 5; Fig. 7D).

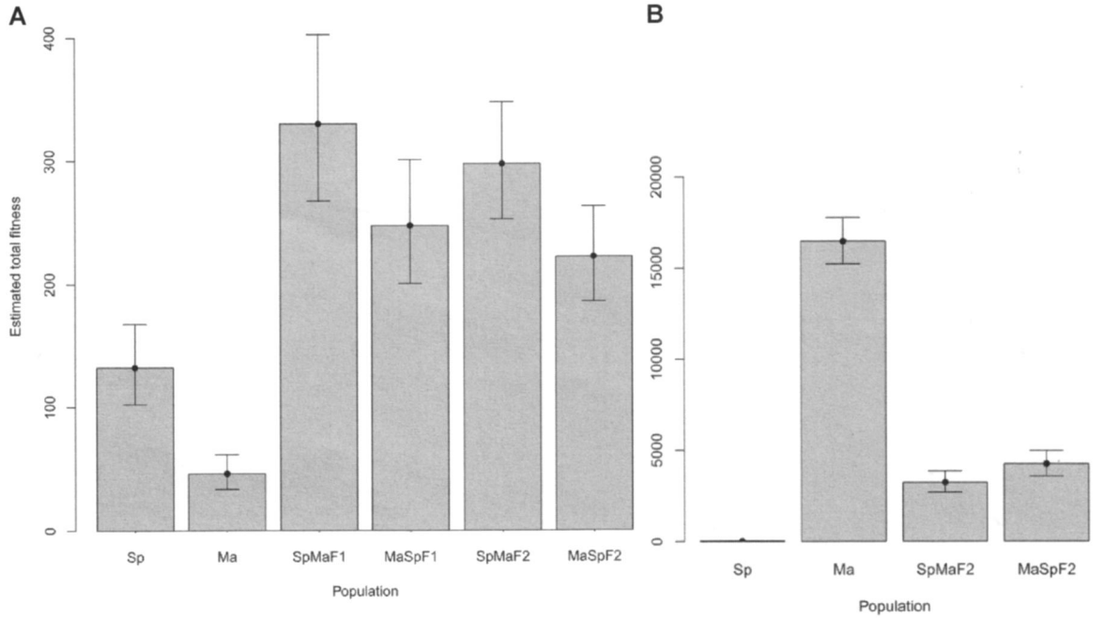

Figure 5. Estimates and ± 1 SE of total fitness from aster models for parental populations Sp Norway and Ma North Carolina US and F1 and F2 hybrid reciprocals (A) over three years in Norway and (B) one year in NC. Note the different scales on the y-axes. See Table 2 for significance tests and Figure one for description of the hierarchical structure of fitness used in the models.

Table 3. Ecological differentiation and gene action in flowering start date and floral display traits (inflorescence length, petal size and total number of flowers). X2 values and significance of likelihood ratio tests of *lmer* models (d.f.=1) are shown for the sites in Norway and NC.

	Year	Nor: Sp-Ma		Nor: Sp- F1		Nor: Sp- F2		Nor: Ma- F1		Nor: Ma- F2		NC: Ma-Sp		NC: Ma- F2		NC: Sp- F2	
		X^2	*P*	X^2	*P*	X^2	*P*	X^2	*P*	X^2	*P*	X^2	*P*	X^2	*P*	X^2	*P*
Fl. start	1st	36.43	<0.0001	15.78	0.0001	16.18	0.0001	13.10	0.0003	13.29	0.0003	15.12	0.0001	5.05	0.0246	3.11	0.0779
date[1,2]		102.51[2]	<0.0001[2]	78.86[2]	<0.0001[2]	102.89[2]	<0.0001[2]	48.22[2]	<0.0001[2]	50.42[2]	<0.0001[2]						
	2nd	2.69	0.1010	0.10	0.7578	0.01	0.9355	2.48	0.1155	2.04	0.1534						
		9.16[2]	0.0025[2]	13.71[2]	0.0002[2]	26.51[2]	<0.0001[2]	12.29[2]	0.0005[2]	9.19[2]	0.002[2]						
	3rd	0.74	0.3880	0.61	0.4344	0.02	0.8930	1.60	0.2057	0.55	0.4593						
		1.72[2]	0.1898[2]	0.27[2]	0.6052[2]	3.83[2]	0.0502[2]	3.81[2]	0.0511[2]	1.99[2]	0.1583[2]						
Infl. length[1]	1st	74.62	<0.0001	48.73	<0.0001	47.67	<0.0001	24.02	<0.0001	26.77	<0.0001	21.32	<0.0001	11.58	0.0007	4.11	0.0427
Petal size		58.72	<0.0001	33.30	<0.0001	25.26	<0.0001	16.51	<0.0001	26.09	<0.0001						
# flowers[1]	1st	7.72	0.0050	8.44	0.0037	19.07	<0.0001	0.20	0.6568	10.01	0.0016						

[1]Square-root transformation.
[2]Kruskal-Wallis test.

Discussion

ADAPTIVE DIFFERENTIATION IN *A. LYRATA*

In the present study of *A. lyrata* populations on different continents, we found support for local adaptation and ecological differentiation between two genetically divergent populations. Habitats of these populations differ in temperature, photoperiod and growing season length. Thus, plants from the Sp population need to be adapted to a cold climate and short growing season, whereas the Ma population experiences heat and intermittent drought and a longer growing season. In their meta-analysis, Leimu and Fischer (2008) found that 45% of reciprocal transplant studies on plants reported such superiority of the local population at both study sites. We know of no studies that have reported local adaptation between diverged plant populations across continents, such as those demonstrated in our study.

An earlier field study on *A. lyrata* revealed local adaptation between three European populations (Leinonen et al. 2009), but the differences in fitness were not as strong as those reported here. Because total fitness was not estimated in hierarchical manner in the previous study, detailed comparisons of overall differences in fitness are difficult to make. Both studies, however, support the hypothesis that adaptive evolution has played a role in population differentiation in this phenotypically diverse species, and that different components of fitness are important in different habitats. Local advantage in the present study was primarily due to higher survival of the local population at the experimental site in Norway and greater reproductive output in NC. Survival and reproductive output showed also contribution to adaptation between the Norwegian and a German population (Leinonen et al. 2009). Such differences in the importance of different components of fitness depending on the environment have been documented; for example, with sunflowers, in which survival was more important for local adaptation in one environment, and reproductive output in another (Sambatti and Rice 2006). In a transplant experiment at different elevations using *Mimulus cardinalis* and *M. lewisii*, growth and reproductive success was affected more in one environment, whereas survival was more important in another habitat (Angert and Schemske 2005).

PHENOTYPIC DIFFERENTIATION IN FLOWERING START AND FLORAL DISPLAY

We found significant ecological differentiation between our study populations. Phenotypic differentiation in flowering start date and inflorescence length was under selection in the direction of the local phenotype at our study site in NC, according to our selection analysis. Such selection in the direction of the local phenotype from an F_2 generation in transplant experiments has been reported in *Gilia* (Nagy 1997). Angert et al. (2008) performed an artificial selection experiment using *Mimulus* hybrids, and found selection for early flowering in high elevation environment. Also in wild

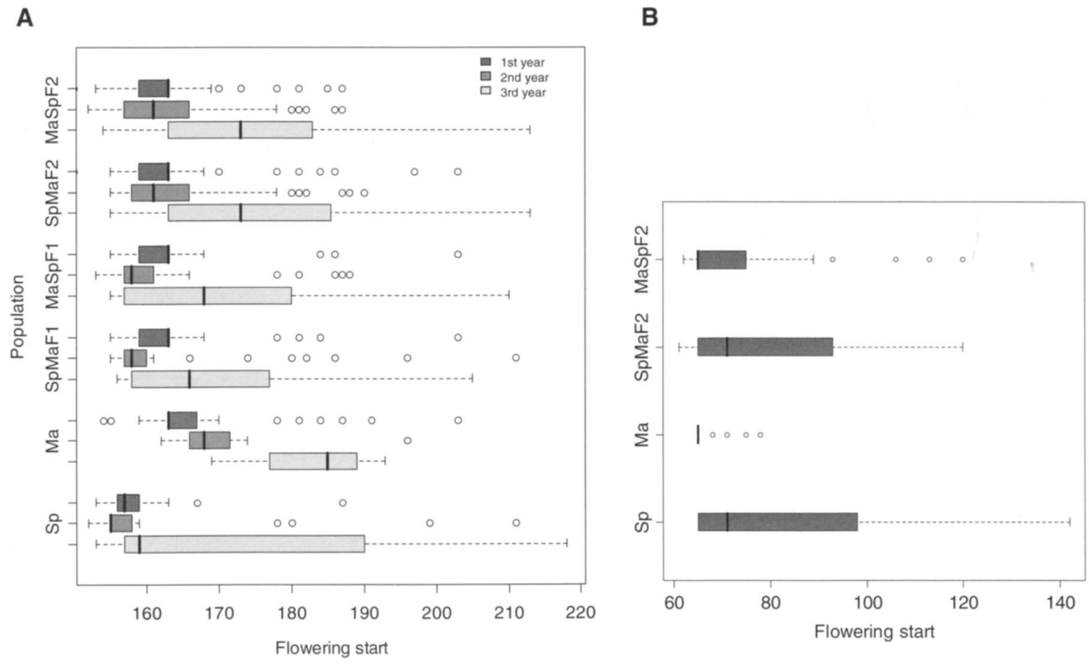

Figure 6. Flowering start (vertical thick line: median, box: upper and lower quartile, dots: outliers) as Julian days from the beginning of the year for parental populations Sp Norway and Ma North Carolina US and F1 and F2 hybrid reciprocals for (A) three years in Norway and (B) one year in NC. Colors indicate data from separate years (see legend). Note the different scales on the x-axes.

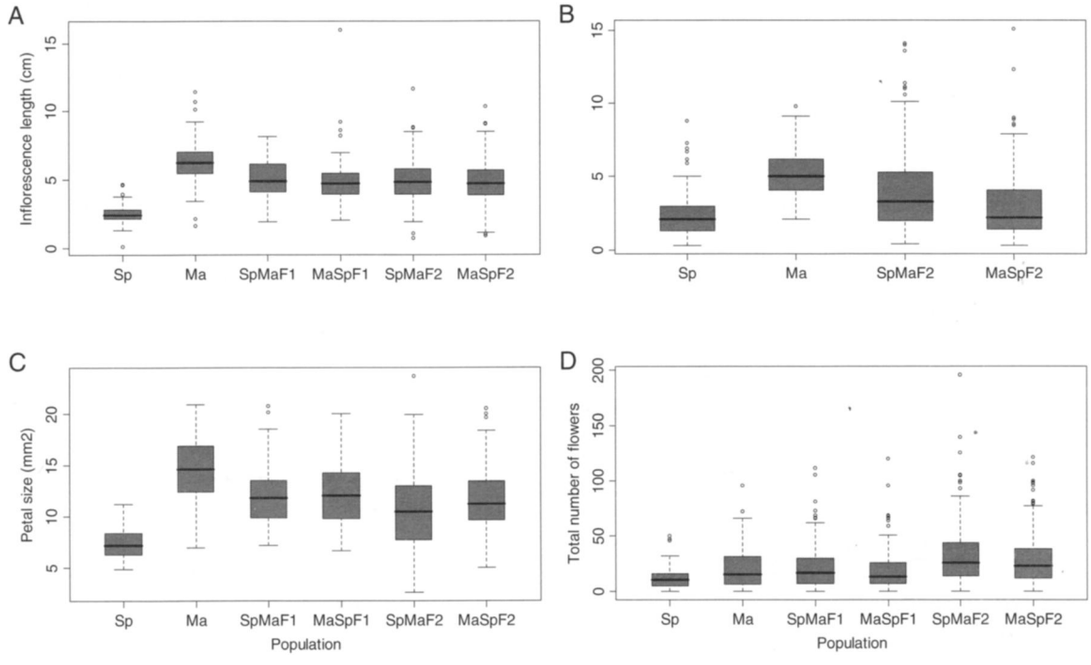

Figure 7. Floral display traits: (A) inflorescence length (cm) in Norway and (B) in NC, (C) petal size (mm^2) in Norway and (D) total number of flowers in Norway (horizontal thick line: median, box: upper and lower quartile, dots: outliers) for parental populations and F1 and F2 hybrid reciprocals.

Table 4. Results of tests for epistatic effects (F2 vs. a 1:2:1 ratio of Sp:F1:Ma contrast) in fitness components, flowering start date and floral display traits at the Norway site, analyzed with contrasts in *lmer* models. Z- value and significance (indicated with bold) is shown for binary traits and 95% confidence intervals for continuous traits.

Trait	Year	Epistasis
Survival	4	+0.08
Flowering%	1st	+1.50
	2nd	+1.30
	3rd	+0.98
	4th	+0.48
# infl.[1]	1st	−0.044; +1.555
	2nd	−0.022; +0.040
	3rd	−0.013; +0.038
Fruits/infl.[1]	1st	−0.061; +0.101
	2nd	−0.090; +0.017
	3rd	−0.071; +0.145
Seeds/fruit[1]	1st	−0.148; +0.025
	2nd	−0.202; +0.086
Fl. start[1]	1st	−0.034; +0.039
	2nd	−0.058; +0.042
	3rd	−0.048; +0.082
Infl. length[1]		−0.031; +0.109
Petal size		−0.658; +0.555
# flowers[1]		**+0.063; +0.421**

[1]Square-root transformation.

barley, flowering time has had an important role in adaptation (Verhoeven et al. 2008). Selection in the direction of the local species or population, in addition to persistence of the phenotypic differences in common growing conditions, suggests that local selection for these traits, and not only genetic drift, have played a role in phenotypic differentiation in our study of two genetically diverged populations after range expansion after the ice age.

Previous studies in controlled conditions have revealed genetically-based flowering start date photoperiodic response differences between populations in *A. lyrata* (Riihimäki and Savolainen 2004; Riihimäki et al. 2005). In our study, both studied populations started flowering early in their home environment. A delayed start of flowering in nonnative environment can be an indication of nonoptimal environmental conditions (e.g., temperature), or inability to respond to specific environmental cues, like photoperiod (Simpson and Dean 2002; Wilczek et al. 2009).

Our analysis of floral display traits showed that the Ma population has longer inflorescences, larger petals and more flowers on average than the Sp population. These differences might have influence on pollinator attraction, for example, if competition for pollinators differs between our study environments. Selection regimes for floral display are known to differ between the Norwegian and a Swedish population (Sandring et al. 2007). Differences in floral display traits could also be related to observed differential allocation to sexual reproduction in these populations.

PATTERNS OF F_2 HYBRID FITNESS

Our results show that hybrid fitness can vary between environments. In our study site in Norway, we found that F_2 hybrid fitness was not lower than fitness of the F_1 generation, as is often predicted (Barton 2001). Such findings have been reported in a recent study on *Iris* (Taylor et al. 2009). These differences are likely due to interactions between genes and environment, but could also be due to relative importance of different components of fitness between sites, such as those observed in this study, and in a previous study in European populations (Leinonen et al. 2009). In spite of relatively high fitness of the hybrids in Norway, especially the survival of the F_2 shows a gradual decrease over the study years, thus potentially lowering hierarchical total fitness in the coming years.

Constraints imposed by differences in the growing season in the two environments also provide a possible explanation for the differences in hybrid performance in the two environments. In NC, flowering and seed set were largely complete by late June, when there were still several months of potential growing season remaining. Genetic mechanisms governing variation in inflorescence production, numbers of flowers produced per inflorescence, and fruit ripening were not limiting factors in NC as a consequence. By contrast, in Norway, the short growing season imposes severe limitations on the time available to complete these processes, increasing the probability that additive genetic effects on reproductive processes will result in nonadditive effects on fitness components when limits imposed by the environment are reached. These limits appear to be exceeded only with complete substitution of Sp alleles with Ma alleles at fitness-related loci, as evidenced by reduced values of some fitness components and survival of Ma plants in Norway. By contrast, partial substitution with non-local alleles may not alter phenology enough to exceed environmental limitations, thus resulting in superior fitness in the hybrids. The potential for some proportion of Ma alleles to improve fitness in Norway could be due to the recent lengthening of growing seasons in northerly environments (ACIA 2004). However, it could also involve the effects of alleles that have evolved in isolation from the Sp population in the Ma lineage, which have not been tested by natural selection in Norway.

Although hybrid fitness showed different patterns in the two environments, at both sites F_2 hybrid fitness was not significantly lower than that of the lower parent at either site. Absence of strong signal of epistatic interactions also suggests that deleterious interactions between nuclear genes might not be crucial for adaptation in our study populations. Recovery of fitness due to the beneficial effects of recombination have been documented, for instance in *Chamaecrista fasciculata* (Erickson and Fenster 2006) and in wild barley (Verhoeven et al. 2004). In *Avena barbata*, hybrid vigor

Table 5. Cytoplasmic effect on phenotypic differentiation. X^2 values and significance of likelihood ratio tests of *lmer* models (d.f.=1) are shown for the sites in Norway and NC.

Trait	Year	Norway				NC	
		F1 reciprocals		F2 reciprocals		F2 reciprocals	
		X^2	P	X^2	P	X^2	P
Survival	4	3.19	0.0743	0.67	0.4122		0.482
Fl.%	1st	0.86	0.3535	0.11	0.7459		0.056
	2nd	0.21	0.6471	0.02	0.8986		
	3rd	0.62	0.4316	1.34	0.2470		
	4th	0.06	0.8110	0.40	0.5255		
# infl.[1]	1st	0.88	0.3494	2.40	0.1213		
	2nd	0.62	0.4293	0.05	0.8302		
	3rd	0.31	0.5800	0.37	0.5454		
Fruits/infl.[1]	1st	0.04	0.8389	1.63	0.2013		0.390
	2nd	0.14	0.7105	0.06	0.8091		
	3rd	4.03	0.0446	1.53	0.2168		
Seeds/fruit[1]	1st	0.21	0.6473	0.79	0.3733		0.006
	2nd	3.70	0.0544	0.17	0.6841		
	3rd	1.76	0.1848	0.47	0.4917		
Fl. start[1]	1st	0.05	0.8231	0.59	0.4415		0.001
	2nd	0.28	0.5943	0.12	0.7251		
	3rd	0.26	0.6104	0.00	0.9757		
Infl. length[1]		1.66	0.1981	1.11	0.2924		
Petal size		1.00	0.3170	12.64	0.0004		
# flowers[1]		1.32	0.2503	3.82	0.0508		

[1]Square-root transformation.

was observed in F_2, but in the F_6 generation, significant hybrid breakdown was found (Johansen-Morris and Latta 2006). Arnold and Hodges (1995) predict also based on their review that hybrid fitness may often be higher than that of at least one of the parents. Lowry et al. (2008a) also found F_1 hybrid vigor in *Mimulus* hybrids only at one of the study sites, but their experiment did not include F_2 hybrids. Evidence for intermediate fitness of advanced-generation hybrids has also been reported also, for example, in experimental "F_2R_1" hybrids of *Mimulus cardinalis* and *M. lewisii* (Angert et al. 2008). In a long-term study of hybrids between *Ipomopsis aggregata* and *I. tenuituba*, the F_2 generation also did not show hybrid breakdown due to nuclear genes, but instead documented cytoplasmic effects on fitness (Campbell et al. 2008).

CYTOPLASMIC CONTRIBUTION TO ADAPTIVE DIFFERENTIATION

We found some evidence for fitness advantage related to the local cytoplasm. In our study populations, the cytoplasmic genomes have differentiated sufficiently to contribute to local adaptation, either through cytoplasmic factors alone or through nuclear–cytoplasmic interactions (Levin 2003). Evolution of cytoplasmic genomes is expected to be faster than that of nuclear genomes, due to the smaller effective population size compared with the diploid nuclear genome. In plants, however, cytoplasmic genes have been observed to evolve on average relatively slowly, because of low mutation rates (Li 1997).

Cytoplasmic components are known to be important in reproductive incompatibilities, but less is known about their role in adaptation. Sambatti et al. (2008) found cytoplasmic origin to be important for survival in hybridizing sunflowers *Helianthus petiolaris* and *H. annuus*. Hybridizing *Ipomopsis* species showed asymmetry (reciprocal differences) in the F_1 generation in the wild (Campbell and Waser 2001; Campbell and Waser 2007; Campbell et al. 2008). Cytoplasmic differences in fitness have been documented also in *Chamaecrista fasciculata* (Galloway and Fenster 2001). In our study, there was also evidence that both nuclear and cytoplasmic genes have contributed to phenotypic differentiation in flowering start date and floral display in our *A. lyrata* populations.

POTENTIAL CONFOUNDING FACTORS

Several caveats should be considered in interpreting our results. Measuring fitness in perennial plants is complicated and requires data over multiple years. Even though our data from Norway includes observations for most traits from only three years, the range of temperature patterns we observed during this period seems likely to be typical of year-to-year variation in summer temperatures. At the site in NC, our estimate of survival is based

on a single year, which may not be indicative of long-term trends on natural sites. However, the differences observed in total fitness between the parental populations were large already after one season at our experimental site in NC.

An even more complete estimate of total fitness at both sites would have required collecting all seeds in all years. Further, the experimental plants should be monitored over their entire lifespan, which is complicated especially in iteroparous perennials, like *A. lyrata*. A more complete measurement and analysis of lifetime fitness has, for example, been performed in experiments using semelparous perennials like *Ipomopsis* (Campbell et al. 2008). In such long-term experiments, it is especially relevant to model fitness as population growth rate, when there is knowledge about factors such as age-specific mortality, that can then be accounted for making interpretations of life history evolution (e.g., Miglia et al. 2005). We think that because we did not have as many years in our dataset, accounting for population growth rate might not be as crucial.

Even though we were not able to estimate lifetime fitness in our experiment, the observed differences in our estimate of total hierarchical fitness between the studied populations are not likely to disappear in the coming years, as the large differences in survival after the second winter between the parental populations in Norway is likely to further increase the relative fitness of the local population with each passing year. Heavy mortality of all populations at the NC site was most likely caused by intermittent drought, in addition to a nematode infestation. Wild plants in the natural population in Mayodan have also shown high mortality after drought periods (D.L. Remington, personal observation), which supports the idea that high reproductive output prior to summer drought stress confers a fitness advantage.

Our study included data from a different number of years at the two study sites. Establishment success of the progeny produced in each year could contribute to fitness differences between transplanted populations, as it has an effect on estimates of population growth. However, because of limitations for planting space in the field, it was not possible to monitor germination and establishment success of the progeny produced by the experimental plants. Also, the resulting progeny would have variable genetic background due to between-population pollinations, which would complicate the conclusions. Our examination of individual fitness components separately, and for each year in Norway, in addition to the comparison of hierarchical total fitness provides further insights on factors influencing longer-term fitness trends for each population in each environment.

In addition, our lack of evidence for hybrid breakdown in the F_2 generation hybrids might also be influenced by the limited study period, as the total lifetime fitness of the hybrids could end up being lower that that of the parental populations. In some species, such as *Iris*, hybrid breakdown occurs in backcross hybrids (Taylor et al. 2009), but these were not studied here. Also, our estimate of the F_2 hybrid fitness was based on a large number of progeny from a single cross, but the F_1 generation was represented by multiple families.

Reciprocal transplant experiments over large geographical distances aim to capture large-scale climatic differences. Our experimental site in NC has the photoperiodic and climatic conditions corresponding to those at the actual site of the population. However, the sandy loam soil at the farm field site used for the NC experiment is unlike the rocky soils in which natural *A. lyrata* populations typically grow in this part of its range, and may be related to the nematode infestation that contributed to the heavy mortality experienced after the end of the flowering season. Planting at the actual site of the Ma population was not possible because the site is dominated by outcrops of bedrock on steep slopes, which would have precluded establishment of a suitable planting grid.

Environmental variation at the planting sites was controlled by randomizing populations in blocks, which was included as a factor in our analyses. Experimental plants at the NC site were randomized at the time of planting, but were pre-grown on different trays in the growth chamber. This is unlikely to have contributed to the observed fitness advantage of the local population, because there were no differences in rosette size between populations in vegetative size at the time of planting (data not shown).

CONCLUSIONS AND FUTURE DIRECTIONS

We found evidence for local adaptation and ecological differentiation in this study. Our results also suggest evolution of different life histories between our study populations, as the Sp plants seemed to allocate fewer resources to sexual reproduction both at the level of reproductive output and floral display than the Ma population. Further studies are needed to find out which environmental and phenotypic characteristics have been responsible for the observed fitness differences. Annual temperature cycles, moisture levels, and photoperiod differ between environments of our study populations, and may be acting as agents of selection. Experimental treatments in controlled conditions would be needed to study the effects of these abiotic factors.

Although we did not find evidence for significant hybrid breakdown and epistasis between nuclear genes in the F_2 generation, our results suggest the possibility of interactions between nuclear and cytoplasmic genomes, as we found association between fitness and cytoplasmic origin. This observed differentiation in nuclear and cytoplasmic genomes also creates potential for reproductive barriers arising as a by-product of adaptation (Coyne and Orr 2004; Sobel et al. 2010), which could lead to genetic incompatibilities between nuclear and cytoplasmic genes. Additional studies have been conducted to examine whether intrinsic incompatibilies (reduced hybrid fertility) have evolved (Leppälä and Savolainen in prep.).

Further evidence for local selection for local quantitative trait locus (QTL) alleles responsible for differences in each fitness component will be evaluated by QTL mapping using the F_2 progeny included in the present study (Leinonen et al. in prep). The relationship between ecological differentiation in flowering start date and floral display and fitness can be inferred by comparing QTL locations. Also, the interactions between the chromosomal segments responsible for the phenotypic differences with the cytoplasm can be examined further.

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