

# Population differentiation and genetic variation inform translocation decisions for *Liatrix scariosa* var. *novae-angliae*, a rare New England grassland perennial

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## Abstract

Augmentation of small rare species populations is sometimes suggested on genetic grounds. However, outbreeding depression via dilution of local adaptation or break-up of genomic coadaptation may occur. These effects depend on the causes of population divergence. Here, we compare genetic measures of population divergence in *Liatrix scariosa* var. *novae-angliae*, a rare New England perennial. We measured  $G_{ST}$ , neutral marker subdivision, and  $Q_{ST}$ , quantitative subdivision of propagule and juvenile plant traits.  $G_{ST}$  was relatively high.  $Q_{ST}$  for leaf shape exceeded  $G_{ST}$ , indicating local adaptation, while  $Q_{ST}$  for other traits fell within or below the  $G_{ST}$  range. Local adaptation appears low for juvenile traits, although the high  $G_{ST}$  cautions against translocation because of potential coadaptation. If translocation is still required, however, donor populations should contain high quantitative genetic diversity. We assess population size and allozyme diversity as predictors of quantitative genetic variation, but find these poor proxies for direct measurement.

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## 1. Introduction

In small, isolated plant populations, genetic drift may lead to the loss of genetic diversity and chance fixation of deleterious alleles, and increased homozygosity can reduce fitness due to inbreeding depression (Barrett and Kohn, 1991; Ellstrand and Elam, 1993). To combat these deleterious effects, augmentation of such populations with individuals from other locations is often proposed (e.g., Griffith et al., 1989). Ideally, augmentation

would result in an influx of genetic variation to the target population, increasing its potential to undergo adaptive evolution (Hamrick and Godt, 1996; Lynch, 1996; Montalvo et al., 1997). In addition, progeny resulting from matings between the resident and translocated individuals may have increased fitness due to genetically based “hybrid vigor” (Waser, 1993; Hufford and Mazer, 2003; Vergeer et al., 2004). However, despite the seeming promise of this conservation strategy, the failure of a number of efforts in the field (Storfer, 1999), as well as increased investigation of population mixing, has highlighted the need for detailed scrutiny of the populations involved (Lynch, 1996; Johnson, 2000; Edmands, 2002).

Initial failure of an augmentation effort can occur if the translocated individuals are insufficiently adapted for survival or reproduction in their new environment (Campbell, 1986; Rehfeldt, 1990, 1991). Perhaps more

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significantly, if translocated individuals interbreed with the resident population, hybrid offspring may have reduced fitness relative to one or both parents, a phenomenon known as outbreeding depression. For managers, it would be advantageous to be able to predict the degree of outbreeding depression so that it can be weighed against the potential benefits of translocation (Ellstrand and Elam, 1993; Montalvo and Ellstrand, 2001; Edmands and Timmerman, 2003). Unfortunately, prediction has been frustrated by variation in outbreeding depression among taxa (Edmands, 2002), generations (e.g., Lynch, 1991; Edmands, 1999; Fenster and Galloway, 2000b), and environments (Lynch and Walsh, 1998; Fenster and Galloway, 2000b; Campbell and Waser, 2001). Empirical estimation of outbreeding depression via taxon-specific, multi-generation field-based studies could provide the necessary data, but because of their logistical demands, few have been attempted.

Alternatively, the relative magnitude of expected outbreeding depression can be estimated by identifying the extent and mechanism of genetic differentiation among candidate populations (Templeton, 1986; Fenster and Galloway, 2000a; Edmands, 2002; Edmands and Timmerman, 2003; Hufford and Mazer, 2003). If a donor population has genetically adapted to a different external environment than the resident population, the hybrid progeny could experience a 50% dilution of the locally adapted genome, resulting in phenotypes that are less well-adapted to the resident environment (the ‘ecological mechanism’ of outbreeding depression) (Montalvo and Ellstrand, 2001). Conversely, a donor population differentiated from the resident as a consequence of processes unrelated to the external environment (but see Fitzpatrick, 2002), including population-specific mutations, genetic drift, and selection on alleles within differing local genetic backgrounds, could also produce hybrid progeny with reduced fitness (the ‘genetic mechanism’ of outbreeding depression) (Price and Waser, 1979). In this mechanism, underdominance, or heterozygote disadvantage, can result from wide divergence in alleles at a single locus (Schierup and Christiansen, 1996). Also, the break-up of coadapted gene complexes initially gener-

ated by restricted gene flow can lead to fitness reductions (e.g., Burton et al., 1999; Edmands, 1999).

Practically, a preliminary understanding of population differentiation can be gleaned by simultaneous assessment of molecular markers and quantitative traits. Molecular markers provide an estimate of the degree of population differentiation via processes unrelated to local adaptation ( $G_{ST}$ ) (Hamrick and Godt, 1990), and thus of the likelihood of outbreeding depression via the ‘genetic mechanism’. As not all markers are selectively neutral (e.g., Berry and Kreitman, 1993; Carter et al., 2000; Lemaire et al., 2000; Watt et al., 2003), care should be taken to employ only those not subject to selection in order to estimate these processes. Furthermore, quantitative trait values can be used to calculate a statistically equivalent metric,  $Q_{ST}$ , which represents population differentiation for these traits (Prout and Barker, 1993; Spitze, 1993). Differentiation of quantitative traits results from the same neutral processes that structure  $G_{ST}$ , as well as natural selection in response to differences in the populations’ local environments. Comparing  $G_{ST}$  and  $Q_{ST}$  then indicates the relative risk of ‘ecological’ outbreeding depression: the greater the magnitude of  $Q_{ST}$  relative to  $G_{ST}$ , the more likely that populations are adapted to different local conditions, and the greater the risk (Table 1). Knowledge of the type(s) of outbreeding depression expected can be combined with geographical and environmental data to highlight the landscape-level processes (e.g., isolation by distance or adaptation to specific environmental factors) relevant to the translocation effort.

If the outcome of these differentiation analyses indicates that substantial outbreeding depression is unlikely, translocation could be a viable conservation strategy (Petit et al., 2001) and the ideal donor population would be one with high molecular and quantitative genetic variation (Hamrick and Godt, 1996; Lynch, 1996; Montalvo et al., 1997; Vergeer et al., 2004). In the absence of resources to evaluate every extant population, the amount of genetic variation could be predicted using the current size of a potential donor population. Theory predicts that smaller populations will have reduced

Table 1

Summary of the type(s) of outbreeding depression expected when populations are mixed for a given combination of  $G_{ST}$  (population subdivision for neutral markers) and  $Q_{ST}$  (population subdivision for quantitative traits) values

$Q_{ST}$ relative to $G_{ST}$	Type of inferred selection across populations	$G_{ST}$	Type(s) of outbreeding depression expected
>	Differentiating (= local adaptation)	High	Genetic, ecological
		Low	Ecological
=	None <sup>a</sup>	High	Genetic
		Low	–
<	Unifying	High	Genetic
		Low	–

High  $G_{ST}$  values (high isolation of populations) indicate an increased risk of ‘genetic’ outbreeding depression, while  $Q_{ST}$  values statistically exceeding  $G_{ST}$  (populations significantly differentiated by natural selection) indicate an increased risk of ‘ecological’ outbreeding depression.

<sup>a</sup> Cannot rule out structure caused by neutral processes.

molecular variation, as well as reduced quantitative genetic variation when census population size is less than 5000–10,000 persistent individuals (Lynch, 1996). However, studies of natural populations have not always borne out these predictions (Waldmann and Andersson, 1998; Podolsky, 2001). Alternatively, quantitative variation might be predicted from molecular markers. Unfortunately, this means of prediction has also been shown to be generally unreliable (Reed and Frankham, 2001), although some exceptions exist (Briscoe et al., 1992). Therefore, empirical investigation of the utility of population size and molecular marker diversity as criteria for selecting donor populations is currently necessary in each taxon of interest.

Here, we demonstrate the use of the  $G_{ST}/Q_{ST}$  comparison method for exploring the forces responsible for population differentiation in Northern blazing star (*Liatris scariosa* var. *novae-angliae*), a rare New England grassland perennial. This taxon is a possible candidate for population augmentation, as 85% of its 82 extant occurrences contain fewer than 500 individuals (Kane and Schmitt, 2001), a precarious situation from a genetic standpoint (Lynch, 1996). Results, supplemented by geographic and environmental data, are then discussed with reference to potential translocation schemes. In addition, we examine the utility of census population size and molecular marker data as proxies for population-level quantitative genetic variation.

## 2. Methods

### 2.1. Study taxon

Northern blazing star (*L. scariosa* var. *novae-angliae* (Lunell): Asteraceae) (Gandhi et al., 2003) is endemic to the northeastern United States, with extant populations in Maine, New Hampshire, Rhode Island, New York, Connecticut, and Massachusetts. It has a status of S1 (endangered) in Maine, New Hampshire, and Rhode Island; S2 (threatened) in New York; S3 (rare) in Connecticut; and S? (special concern) in Massachusetts (Kane and Schmitt, 2001). Of 214 reported historical and extant occurrences of this plant, approximately 82 persist today (Kane and Schmitt, 2001).

Northern blazing star is an herbaceous, iteroparous perennial. Other *Liatris* species are predominantly outcrossing (e.g., *L. helleri*) (Godt and Hamrick, 1995), and preliminary data (Kane, 2001; E.V., M. Vadeboncoeur and E. Boyd, unpublished data) suggest that Northern blazing star seeds produced by selfing are not viable. Propagules (achene-pappus units) are wind-dispersed. Northern blazing star is usually found in early successional habitats characterized by sandy, nutrient-poor soils, including sandplain grasslands, xeric heathlands, roadsides, and pine barrens (Hamilton, 1991; Collins,

1999). Currently, New England grasslands are declining from suppression of fire and disturbance, which has resulted in successional takeover of these sites (Vickery and Dunwiddie, 1997). Northern blazing star is threatened by this habitat loss, as well as by land development, propagule predation, deer grazing, and low juvenile recruitment (Kane, 2001; Kane and Schmitt, 2001).

### 2.2. Study sites

To select sample sites, we listed occurrences where sampling would be legally permitted on the basis of population size and landowner consent, and then randomly chose sites from this list. Unfortunately, this restricted our potential sampling area to a subset of the plant's full range (Fig. 1). Our twelve sample sites included four occurrences on Block Island, RI (AIR, FSP, GIB, TNP); two on Nantucket, MA (MIG, NLB); two on Martha's Vineyard, MA (KAT, CHP); one on the Massachusetts mainland (WBI); two on the Connecticut mainland (NHN, NWT); and one on the Maine mainland (KBP) (Fig. 1). Appropriate permission agreements were obtained from state agencies and landowners prior to sampling.

### 2.3. Allozymes

We collected leaf tissue from 24 individuals per population in July, 2002. Leaf samples were placed in Ziploc bags and shipped in ice-filled coolers to the laboratory

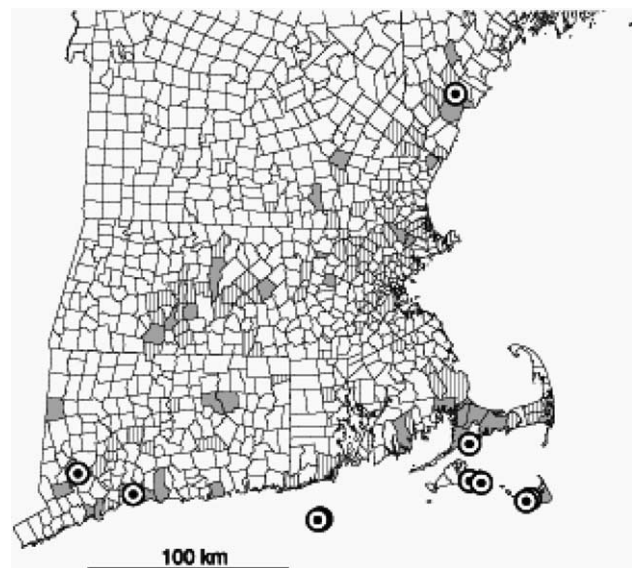


Fig. 1. GPS locations of study sites overlaid on *Liatris scariosa* var. *novae-angliae* distribution map (Kane and Schmitt, 2001). States in New England, USA containing historical and extant occurrences of this taxon occurs are shown, with town boundaries within each state outlined. Shading denotes towns containing extant occurrences, while hatching indicates historic occurrences.

of J.L. Hamrick (Athens, GA). The tissue was crushed using a mortar and pestle, adding liquid nitrogen and a crushing buffer (Wendel and Parks, 1982) to extract the enzymes. Extracts were absorbed onto wicks cut from Whatman 3MM chromatography paper, placed in 96-well microtest plates, and stored at  $-70^{\circ}\text{C}$  until they were loaded onto 10% horizontal starch gels for electrophoresis. We used 13 enzyme stains to resolve 24 loci. Loci resolved were fluorescent esterase (*Fe-1*, *Fe-2*, *Fe-3*, *Fe-4*, *Fe-5*, and *Fe-6*), phosphoglucosomerase (*Pgi-1*, *Pgi-2*, and *Pgi-3*), aspartate aminotransferase (*Aat-1* and *Aat-2*), diaphorase (*Dia-1*), menadione reductase (*Mnr-1*), leucine aminopeptidase (*Lap-1*), uridine diphosphatase (*Ugpp-1* and *Ugpp-2*), triose-phosphate isomerase (*Tpi-1* and *Tpi-2*), phosphoglucosomutase (*Pgm-1*), malate dehydrogenase (*Mdh-1*), 6-phosphogluconate dehydrogenase (*6Pgd-1* and *6Pgd-2*), and isocitrate dehydrogenase (*Idh-1* and *Idh-2*). The buffer systems used were system 6 (*Fe-1*, *Fe-2*, *Fe-3*, *Pgi*), system 7 (*Mnr*, *Aat*, *Dia*), system 10 (*Lap*, *Ugpp*), and a modified system 8 (*Fe-4*, *Fe-5*, *Fe-6*, *Tpi*, *Pgm*) (Soltis et al., 1983). A morpholine citrate (MC) buffer system (Kephart, 1990) was used for *Mdh*, *6Pgd*, and *Idh*. Enzyme stains followed Soltis et al. (1983), with the exception of the stains for diaphorase and menadione reductase (Cheliak and Pitel, 1984).

Genetic parameters were calculated for each population as well as for the species (following Hamrick and Godt, 1996) using LYNPROG software provided by J.L. Hamrick. The 95% confidence interval for  $G_{ST}$  was calculated using the distribution of  $G_{ST}$  values for each locus. Each locus was tested for significant population structure using a  $\chi^2$  test, with final significance evaluated after accounting for multiple tests ( $\alpha = 0.0026$ ).

Because allozyme neutrality is of key importance to the  $G_{ST}/Q_{ST}$  comparison, we tested this assumption in several ways. First, a Ewens–Watterson test was applied to each locus. This test detects selection on individual loci in the form of heterozygote advantage, heterozygote disadvantage, or the presence of deleterious alleles (Watterson, 1978), and was implemented using the algorithm of Manly (1985) (1000 permutations) in the POPGENE 1.32 software package (Yeh et al., 1998). In addition, we collected climate and soil data from the study sites and assessed the relationship of these data to allozyme allele frequencies. The following climate data (1952–2001) were obtained from the National Oceanic and Atmospheric Administration (NOAA) weather station nearest each population (<http://cdo.ncdc.noaa.gov/plclimprod/plsql/poemain.poe>): annual cooling degree days, annual heating degree days, minimum temperature in January, maximum temperature in July, monthly mean temperature, and total monthly precipitation. Data were averaged over all years. For the soil assessment, approximately 250 cm<sup>3</sup> of soil were obtained from each study site at the time of seed collection and

sent to the University of Massachusetts Amherst Soil and Plant Tissue Testing Laboratory (<http://www.umass.edu/plsoils/soiltest>) for analysis. The following data were obtained: organic matter content, pH, buffer pH, extractable nutrients, extractable heavy metals, extractable Al, cation exchange capacity, % base saturation, and soluble salt concentration. Climate and soil data were separately subjected to principal components analysis using PROC PRINCOMP in SAS version 8.0 SAS (1999), and principal components with eigenvalue >1 were retained (the “latent root criterion,” Hair et al., 1998). We then regressed the frequency of each allozyme allele on each climate and soil principle component using PROC GLM in SAS. For correlations significant at the 0.05 level and not driven by a single point, the entire locus to which the suspect allele belonged was removed from the allozyme data set, and the  $G_{ST}$  range re-calculated. Although a relationship between the frequency of an allozyme allele and climatic or soil conditions does not necessarily indicate selection on that allele, this statistically and conceptually conservative approach allowed us to explore the potential impact of such selection on our  $G_{ST}$  range, and the implications for its comparison with  $Q_{ST}$ .

#### 2.4. Propagule collection and measurement

As described by Gravuer et al. (2003), we collected Northern blazing star propagules from mid-September through early November, 2001. We also censused the reproductive individuals in each population, counting a smaller area and extrapolating for populations >1000 individuals. At each sample site, we collected 10 propagules from each of 10 plants, selecting plants at random from a transect covering the entire apparent area of each population. In the laboratory, we weighed each propagule to the nearest 0.1 mg and measured each achene length, achene width, and longest pappus bristle using digital photographs and Adobe Photoshop 6.0 (Adobe Systems, 2000).

#### 2.5. Morphological trait measurement and analysis

On January 10, 2002, propagules were sown into 8.9 cm square pots containing a mixture of 60% coarse-grained sand and 40% MetroMix (Scotts-Sierra Horticultural Products Co., Marysville, OH, USA). Pots were fully randomized on two benches in a heated greenhouse at Brown University. We censused the pots every other day, recording plant emergence date and the date of leaf initiation for each leaf produced. We also measured the length and width of cotyledons, allowing a minimum of 10 days between plant emergence and cotyledon measurement to ensure full cotyledon expansion. On March 28 and May 12, 2002, we measured the length and width of all leaves on all plants to calculate esti-

mates of leaf shape and growth rate for two time intervals (interval 1: January 10 to March 28; interval 2: March 28 to May 12). In late May 2002, the juvenile plants were moved to a field plot in anticipation of including the following year's adult characters in the analysis. However, extreme summer and winter conditions resulted in high mortality, reducing sample size below a statistically valid range.

For statistical analysis, traits were calculated and transformed as follows. Growth rate was calculated as  $[\ln(\text{total leaf area})/\text{days emerged}]$ . Development rate was calculated as  $[\text{total number of leaves}/\text{days emerged}]$ . Each of the two time intervals was analyzed separately for both growth rate and development rate. Leaf shape (of the longest leaf) was calculated as the residuals of a regression of length on width (PROC GLM), and cotyledon shape was calculated as the ratio of length to width. Leaf shape on each of the two measurement dates was assessed separately. To meet the assumptions of the statistical analysis, propagule mass and cotyledon area were square-root transformed, and achene width, cotyledon shape, and development rate 1 were log-transformed. All other traits were left untransformed.

Because maternal effects can bias estimates of genetic variation, especially for traits early in life (Schaal, 1984; Roach and Wulff, 1987), we controlled for the effect of initial propagule mass on subsequently measured traits (Paige and Heschel, 1996). Although this correction does not take into account all potential sources of maternal effects, its role in mediating maternal influence on offspring traits is well-supported (Roach and Wulff, 1987). We regressed all trait values on initial propagule mass for each individual using SAS PROC GLM. As cotyledon area showed a significant relationship with propagule mass, we conducted subsequent analyses on both its raw values and on the residuals resulting from the propagule mass regression. No other trait was significantly related to initial propagule mass.

We used a nested ANOVA (SAS PROC GLM) to evaluate family and population effects on quantitative traits, with both population and family nested within population as random effects. To obtain  $Q_{ST}$  values and their standard errors, we used a software program provided by M. Lynch. This program calculated variance component estimates among populations ( $V_{\text{among}}$ ) and within populations ( $V_{\text{within}}$ ) using a similar nested ANOVA. Standard errors were computed using a procedure based on the delta method (Lynch and Walsh, 1998). Since *L. scariosa* var. *novae-angliae* is predominantly outcrossed, it is likely that our sibships represented a mix of full and half siblings. We modified the software to calculate estimates under both potential relationships, using  $Q_{ST} = V_{\text{among}}/(V_{\text{among}} + 4V_{\text{within}})$  for full sibs and  $Q_{ST} = V_{\text{among}}/(V_{\text{among}} + 8V_{\text{within}})$  for half sibs (Yang et al., 1996). We used a clonal model

( $Q_{ST} = V_{\text{among}}/(V_{\text{among}} + 2V_{\text{within}})$ ) for pappus length because it is maternal tissue.  $Q_{ST}$  was determined to differ from  $G_{ST}$  when the 95% confidence intervals for the two estimates did not overlap. Finally, we tested for associations between population mean quantitative trait values (response variable) and the climate and soil principal components (explanatory variable) with SAS PROC GLM. Significant relationships would suggest an influence of climate and soil differences among sites on selection patterns detected in the  $G_{ST}/Q_{ST}$  comparison.

## 2.6. UPGMA clustering and isolation by distance assessment

For allozymes, Nei's genetic distance measures were calculated (Nei, 1972) using LYNPROG software. Morphological distances between populations (Mahalanobis  $D^2$ ) were computed using SAS PROC CANDISC. Separate analyses were performed for plant and propagule traits. We performed UPGMA clustering on the distance values (allozymes) and the square root of the Mahalanobis  $D^2$  values (morphological traits) using MEGA software, version 2.1 (Kumar et al., 2001).

Geographical structuring of allozymes and trait groups was analyzed with a Mantel test (1000 permutations) in the R software package (Casgrain and Legendre, 2002). Allozyme and trait matrices were those used for UPGMA clustering. The geographic distance matrix was constructed using site GPS measurements. For each set of matrix comparisons, we applied a Bonferroni correction to account for multiple tests ( $\alpha = 0.017$  for each test).

## 2.7. Effects of population size and relationships among genetic diversity measures

We determined the association between census population size (hereafter, "population size"), molecular variation, and quantitative genetic variation for each of the common-garden plant traits. Quantitative genetic variation was quantified as both the coefficient of genetic variation ( $CV_G$ ) and broad-sense heritability ( $h^2$ ) (Houle, 1992; Lynch, 1996). To conduct the analyses,  $CV_G$  was calculated as  $[(\sqrt{V_G}/\text{trait mean}) \times 100]$  and subsequently divided by trait dimensionality (Houle, 1992). Under the full-sib assumption,  $V_G$  was estimated as twice the "family within population" variance component from SAS PROC VARCOMP (REML method). Broad-sense heritability and its standard error were computed using a nested ANOVA and the delta method, respectively, with the computer program provided by M. Lynch. When negative variance components resulted, we set  $h^2$  equal to 0. We also calculated 95% confidence intervals for the heritability estimates and recorded the number of traits in each population for which this interval

did not overlap zero, as a measure of the number of traits containing significant broad-sense genetic variation.

The association between population size and each set of metrics was assessed using the Spearman option of PROC CORR because the data did not meet the assumptions of a parametric statistical analysis. In addition, we assessed the relationship between population allozyme diversity (=expected heterozygosity,  $H_e$ ) and quantitative genetic variation of each trait, again using SAS PROC CORR option Spearman. The prediction of  $H_e$  by log-transformed population size was conducive to a parametric analysis, and SAS PROC GLM was used. Finally, because the relationship between population size and quantitative genetic variation may become asymptotic at effective population sizes above 1000 (=census size of approximately 5000–10,000) (Lynch, 1996), we repeated this set of analyses first excluding populations >10,000 (KBP), then excluding populations >5000 (AIR, TNP, KBP). For each analysis, we applied a Bonferroni correction based on the number of traits assessed (7 for  $CV_G$  analyses, 10 for  $h^2$  analyses).

### 3. Results

#### 3.1. Allozyme structure and neutrality

$G_{ST}$  values for polymorphic loci ranged from 0.038 to 0.471, with 17 of 19 loci exhibiting significant structure (Supp Table 1).  $G_{ST}$  was equal to 0.212, with a 95% confidence interval of 0.160–0.265. This  $G_{ST}$  value is moderate when compared to species with similar life history characteristics (Hamrick and Godt, 1990). Gene flow

among populations was low to moderate, with an  $N_m$  value of 0.32 using Slatkin's method of rare alleles (Slatkin, 1985) and a value of 0.93 using  $G_{ST}$  (Wright, 1951). A high number of private alleles, 13, were found in the total population sample.

In the Ewens–Watterson test, all loci were neutral at the 0.05 level, indicating no evidence for selection in the form of heterozygote advantage, heterozygote disadvantage, or effects of deleterious alleles (Watterson, 1978). Allele frequencies were significantly correlated with climate and soil principal components approximately twice as often (at  $p < 0.05$ ) as would be expected by chance (51 vs. 23). However, inspection revealed that many relationships were driven by a single point, in large part due to the high prevalence of rare alleles in the data set. When these instances were eliminated, five suspicious loci remained: *Aat-1*, *Lap-1*, *Pgi-3*, *Mdh-1*, and *Fe-6*. The  $G_{ST}$  values for these loci were not extreme compared to the full range of values (Supp Table 1). When these loci were eliminated, the recalculated  $G_{ST}$  was 0.218, with a 95% confidence interval of 0.151–0.284.

#### 3.2. Quantitative trait structure

Most of the traits differed significantly among populations and among families within populations (Supp Table 2). However, cotyledon shape, cotyledon area (accounting for propagule mass), development rate 1 and 2, and leaf shape 2 differed among populations but not families, whereas pappus length did not differ significantly among populations but did differ significantly among families (Supp Table 2). Only growth rate 2 did not differ significantly within or among populations (Supp Table 2).

Table 2  
 $Q_{ST}$  values and significance

Trait	Full sib assumption				Half sib assumption				
	$Q_{ST}$	95% Lower	95% Upper	Comp. to $G_{ST}$	$Q_{ST}$	95% Lower	95% Upper	Comp. to $G_{ST}$	
Propagule mass	0.092	0.018	0.167		0.048	0.009	0.087	–	
Achene length	0.069	0.006	0.132	–	0.035	0.002	0.068	–	
Achene width	0.050	–0.007	0.107	–	0.026	–0.003	0.055	–	
Days to emergence	0.145	0.008	0.282		0.078	0.004	0.153	–	
Cotyledon shape	0.545	0.218	0.872		0.374	0.151	0.597		
Cotyledon area	0.396	0.231	0.561		0.247	0.145	0.349		
Cotyledon area (pmr)	0.569	0.351	0.787	+	0.398	0.247	0.549		
Growth rate 1	0.075	–0.072	0.222		0.039	–0.037	0.115	–	
Development rate 1	0.787	–0.489	2.063		0.648	–0.405	1.701		
Development rate 2	0.088	–0.190	0.366		0.046	–0.099	0.191		
Leaf shape 1	0.316	0.100	0.532		0.188	0.061	0.315		
Leaf shape 2	0.711	0.446	0.976	+	0.552	0.348	0.756	+	
	Clonal model								
Pappus length	0.022	–0.041	0.085	–					

The 95% confidence interval for each trait's  $Q_{ST}$  value was compared to that for  $G_{ST}$  (0.160–0.265). When intervals did not overlap,  $Q_{ST}$  was considered significantly larger (+) or smaller (–) than  $G_{ST}$ . For pappus length, a maternal tissue, we used a clonal rather than a sibling model to calculate  $Q_{ST}$ . Pmr = propagule mass residuals, as propagule mass was significantly related to this trait and was factored out to minimize maternal effects. Under both assumptions,  $Q_{ST}$  for leaf shape 2 fell above  $G_{ST}$  (= differentiating selection), and achene length, achene width, and pappus length fell below  $G_{ST}$  (= unifying selection).

In general, climate and soil principal components did not show significant relationships to population mean trait values, with 6 of 90 comparisons significant at the 0.05 level as compared to 4.5 expected by chance.

### 3.3. $G_{ST}/Q_{ST}$ comparison

Under the full sib assumption,  $Q_{ST}$  values ranged from 0.050 (achene width) to 0.787 (development rate 1) (Table 2, Supp Table 3). Traits with  $Q_{ST}$  greater than  $G_{ST}$  were cotyledon area (accounting for propagule mass) and leaf shape 2. Traits with  $Q_{ST}$  less than  $G_{ST}$  were achene length and achene width. Under the half sib assumption,  $Q_{ST}$  values ranged from 0.026 to 0.648 (Table 2, Supp Table 3). Leaf shape 2 was the only trait with  $Q_{ST}$  greater than  $G_{ST}$ , while traits with  $Q_{ST}$  less than  $G_{ST}$  included propagule mass, achene length, achene width, days to emergence, and growth rate 1. Pappus length, for which a clonal model was assumed, also had  $Q_{ST}$  less than  $G_{ST}$ . Using  $G_{ST}$  calculated with suspicious loci excluded, the results were virtually unchanged; only  $Q_{ST}$  for days to emergence under the half

sib assumption now fell within, not below, the  $G_{ST}$  range.

In summary, under both sibling assumptions and with either  $G_{ST}$ ,  $Q_{ST}$  for leaf shape 2 was greater than  $G_{ST}$ , suggesting differentiating selection (Table 1), and  $Q_{ST}$  for achene length, achene width, and pappus length were less than  $G_{ST}$ , suggesting unifying selection (Table 1).

### 3.4. UPGMA clustering and isolation by distance

Moderate differences could be noted in the population groupings generated by allozymes, propagule traits, and plant traits (Figs. 2–4). Allozymes exhibited slight, although statistically insignificant, geographic structure (isolation by distance) (Mantel’s  $r = 0.243$ ,  $p = 0.041$ ). However, neither propagule nor plant morphological traits were structured geographically (propagules: Mantel’s  $r = -0.043$ ,  $p = 0.433$ ; plants: Mantel’s  $r = -0.078$ ,  $p = 0.417$ ). Morphological distance from propagule traits, but not from plant traits, exhibited significant correlation with allozyme distance (propagules vs. allozymes: Mantel’s  $r = 0.511$ ,  $p = 0.002$ ; plants vs. allozymes: Mantel’s  $r = 0.195$ ,  $p = 0.119$ ). Propagule and plant morphological distances were not significantly related after correcting for multiple tests (Mantel’s  $r = 0.335$ ,  $p = 0.029$ ).

### 3.5. Diversity measures and population size

The sampled populations had fairly high levels of molecular genetic diversity (Supp Table 4), especially compared to species with similar geographic range (Hamrick and Godt, 1990). Quantitative genetic diversity, however, varied considerably among traits, with

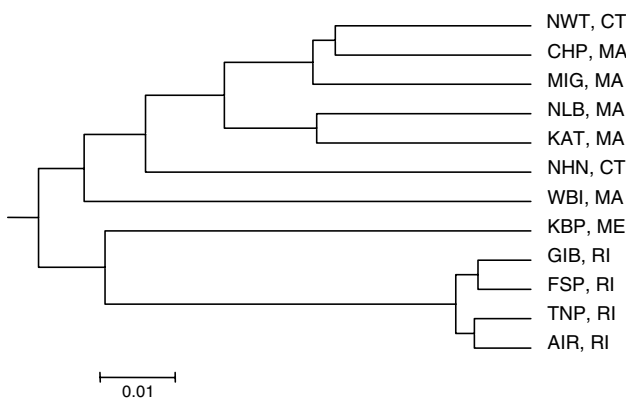


Fig. 2. UPGMA tree based on 19 polymorphic allozyme loci. Units are Nei’s genetic distance (Nei, 1972).

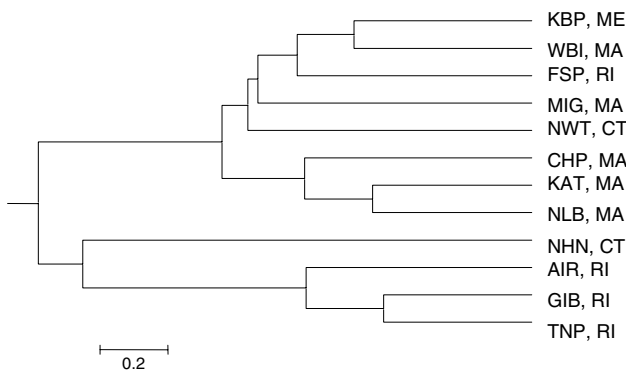


Fig. 3. UPGMA tree based on propagule morphology traits (propagule mass, achene length, achene width, pappus length, and germination success). Units are  $\sqrt{(\text{Mahalanobis } D^2)}$ .

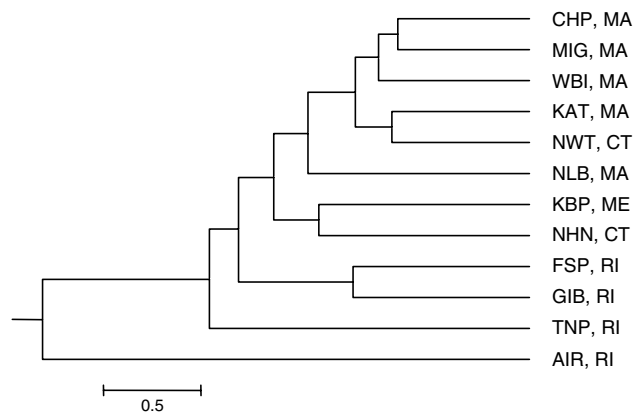


Fig. 4. UPGMA tree based on plant morphology traits (days to emergence; cotyledon length and width, both controlled for differences in initial propagule mass; number of leaves at two time points; total leaf area at two time points; longest leaf length and width at two time points, both controlled for differences in initial propagule mass). Units are  $\sqrt{(\text{Mahalanobis } D^2)}$ .

some traits in some populations having negligible quantitative genetic diversity (Supp Table 5).

Overall, population size marginally predicted allozyme diversity ( $r^2 = 0.297$ ,  $p = 0.067$ ), but neither population size nor allozyme diversity was strongly related to quantitative genetic variation. Note, however, that low statistical power ( $n = 12$ ) may have limited our ability to detect these relationships. After Bonferroni correction, population size was not related to either  $CV_G$  or  $h^2$  for any trait. This pattern held for both the full set of populations and when large populations were omitted. Likewise, the number of traits with significant broad-sense genetic variation in each population was not strongly predicted by population size (Spearman's correlation coefficient = 0.525,  $p = 0.080$ ). Allozyme diversity also generally failed to predict quantitative genetic diversity. Only cotyledon area showed some relationship to  $H_e$ , with cotyledon area uncorrected for propagule mass showing a significant relationship between  $CV_G$  and  $H_e$  (Spearman's correlation coefficient = 0.762,  $p = 0.004$ ), and cotyledon area corrected for propagule mass showing a significant relationship between  $h^2$  and  $H_e$  (Spearman's correlation coefficient = 0.755,  $p = 0.005$ ). None of the other variation measures were related to  $H_e$ , and there was no evidence for confounding by asymptotic relationships.

## 4. Discussion

### 4.1. $G_{ST}/Q_{ST}$ comparison

For all but two of the characters we measured,  $Q_{ST}$  was equal to or lower than  $G_{ST}$ ; thus strong differentiating selection does not appear to have shaped morphological population differences. If these traits contribute significantly to the adaptation of populations to their environments, this result predicts that post-translocation outbreeding depression via the 'ecological mechanism' should be relatively low. A result of  $Q_{ST}$  less than or equal to  $G_{ST}$  differs from the findings of recent reviews (Merilä and Crnokrak, 2001; McKay and Latta, 2002), in which  $Q_{ST}$  usually exceeded  $G_{ST}$  in natural populations. However, among studies of rare species, our results seem more typical; Waldmann and Andersson (1998) found  $Q_{ST}$  similar to or slightly lower than  $G_{ST}$  in the rare *Scabiosa canescens*, and Petit et al. (2001) found  $Q_{ST}$  less than  $G_{ST}$  for juvenile traits in two rare species, *Centaurea corymbosa* and *Brassica insularis*, and a trend for  $Q_{ST}$  less than  $G_{ST}$  for adult traits of *B. insularis*.

Petit et al. (2001) proposed that the "restricted ecological niche" of rare species could cause them to experience homogenous selection pressures, resulting in low  $Q_{ST}$ . As *L. scariosa* var. *novae-angliae* depends on a rare,

edaphically unique habitat (Mehrhoff, 1997; Collins, 1999; Vickery, 2002), it is likely more restricted ecologically than most organisms examined to date, possibly contributing to its lower  $Q_{ST}$ . Alternatively, Waldmann and Andersson (1998) suggested that the low  $Q_{ST}$  values may indicate reduced responsiveness to local selection pressures. While our results did not suggest particularly low levels of genetic variation, the small size of many of our populations may result in reduced effectiveness of selection relative to random genetic drift. Additionally, attributes of *L. scariosa* var. *novae-angliae* unrelated to rarity might underlie this pattern. The potential for metapopulation dynamics (Kane and Schmitt, 2001; E. Steinauer, MA Audubon Society, pers. comm.), as well as the plant's early successional, temporally variable habitat, may make local adaptation to specific sites a poor strategy (Galloway and Fenster, 2000; Campbell and Sugamo, 1989). Additionally, the propagule traits in particular may face allometric, ecological, or phylogenetic constraints to adaptive differentiation (Harper et al., 1970; Matlack, 1987; Gravuer et al., 2003).

Although most traits did not show a strong signature of differentiating selection,  $Q_{ST}$  for leaf shape was higher than the  $G_{ST}$  range. Differentiation in leaf shape could result from the mowing management of several of the populations, as these populations were more prostrate and produced shorter, wider leaves in the greenhouse. Studies on other species have demonstrated adaptations to mowing in development rate (van Tienderen and van der Toorn, 1991a,b), internode length and number (Zopfi, 1998), leaf number and plant stature (McKinney and Fowler, 1991), and growth habit (Linhart and Grant, 1996). Practically, consideration of leaf shape when planning a translocation may avoid maladapted leaf forms. A morphological distance tree based on leaf shape that could aid planning is presented in Supplemental Figure 1.

Our comparison of  $G_{ST}$  and  $Q_{ST}$  allows useful inferences as to the likelihood of 'ecological' outbreeding depression and aids in delimiting population combinations that would minimize its impact. However, one caveat is that we only measured juvenile plant traits, and it is possible that traits expressed later in the life cycle exhibit greater differentiation. Two previous studies in plants (Tonsor et al., 1993; Bonnin et al., 1996) found that population differentiation increased as the life cycle progressed, although another reported mixed results (Gilmartin et al., 1986). Including adult traits would increase the likelihood that fitness-related traits had been captured, and is clearly necessary before final conclusions can be reached. Still, for this early successional plant, many of the traits we measured should be important for survival of juveniles (e.g., growth and development rates), and would be expected to reflect adaptive population differentiation if it existed. Secondly, our propagule traits are not proper  $Q_{ST}$  mea-

tures due to their field collection (Crnokrak and Merilä, 2002); maternal effects undoubtedly influenced the patterns of variation detected. Different types of maternal effects (Roach and Wulff, 1987) would be expected to have opposing influence on  $Q_{ST}$  (Podolsky and Holtsford, 1995; Waldmann and Andersson, 1998; Petit et al., 2001), with maternal genotype effects more likely to cause a downward bias through inflation of  $V_G$  and maternal environment effects more likely to cause an upward bias. However, as studies of natural populations have shown that environmental maternal effects often predominate in influencing traits of propagules (Antonovics and Schmitt, 1986), our propagule  $Q_{ST}$  values are probably overestimates, suggesting that eliminating maternal effects would not change our finding that  $Q_{ST} < G_{ST}$ .

Limits common to most  $G_{ST}/Q_{ST}$  studies are also worth considering. First, for the  $G_{ST}$  value, we assumed selective neutrality of the allozymes. The neutral results of the Ewens–Watterson test, the low number of alleles related to climate and soil parameters, and the robustness of the  $G_{ST}$  range to deletion of these loci all suggest that direct selection has been minimal. Furthermore, considering a large number of loci should minimize problems of linkage disequilibrium with other genes under selection (Podolsky and Holtsford, 1995). Second, our use of broad-sense genetic variance to calculate  $Q_{ST}$  could create bias. Maternal effects (Podolsky and Holtsford, 1995; Waldmann and Andersson, 1998; Petit et al., 2001), dominance (Podolsky and Holtsford, 1995), and epistasis (Lynch, 1994; Phillips et al., 2000) could each contribute to skewed  $Q_{ST}$  values relative to those which would be obtained using narrow-sense genetic variance. While we controlled for maternal effects resulting from differences in initial propagule mass, we did not control for these other factors. Third, we assumed that genetic variation as measured in the greenhouse is similar to that measured in the field (Podolsky and Holtsford, 1995), which may not be the case (Merilä and Crnokrak, 2001). Other important assumptions have been discussed recently (Yang et al., 1996; Hendry, 2002).

#### 4.2. Patterns of allozymes and morphological traits

Although comparing  $G_{ST}$  and  $Q_{ST}$  suggests relatively benign ‘ecological’ outbreeding depression, the ‘genetic mechanism’ must still be considered. Although simulation study indicates that the ‘ecological mechanism’ may create more severe outbreeding depression sooner, the ‘genetic mechanism’ may produce a longer-lasting effect on progeny fitness (Edmands and Timmerman, 2003), and is thus no less of a concern. Our allozyme data indicated that populations were somewhat isolated from one another, with gene flow on the order of 0.32–0.93 migrants per generation. As restricted migration be-

tween populations, especially in combination with small population size, may allow the evolution of ecologically significant levels of genomic coadaptation (Burton et al., 1999; Edmands, 1999; Fenster and Galloway, 2000b), we recommend crossing trials among target and donor populations before ‘genetic’ hybrid breakdown can be ruled out.

Like another rare plant study (Petit et al., 2001), we found that neither propagule nor plant morphological distances exhibited an overall correlation to geographic distance, while allozyme allele frequencies showed slight geographic structure. Isolation by distance in the allozymes is theoretically expected given the low to moderate levels of gene flow that we detected among populations (Wright, 1951; Schaal, 1974). The fact that restricted gene flow does not generate a similar pattern in quantitative traits supports the inference that natural selection (unifying or differentiating) is acting on at least some of these traits. In addition, our comparative analysis of allozyme and trait group structure confirmed that these data sets can provide distinct, complementary information, as reported by other authors (e.g., Long and Singh, 1995; Podolsky, 2001; Knapp and Rice, 1998; but see, Steinger et al., 2002). Geographical patterns might also be expected if abiotic variables that varied on the spatial scale we sampled were an important instrument of differentiating natural selection (e.g., Jaramillo-Correa et al., 2001), but we did not find evidence for this in either allozymes or quantitative traits. Rehfeldt (1999) demonstrated that climatic differentiation patterns can break down in species with isolated, disjunct populations. Climate might be more important for life history and phenological traits.

#### 4.3. Predicting genetic diversity

Relationships among population size and genetic diversity were mixed. We found a marginal relationship between allozyme diversity and population size, consistent with theoretical expectations (Wright, 1951) and some previous empirical findings (Ellstrand and Elam, 1993; Frankham, 1996). A relationship between population size and diversity of neutral markers was also found in a RAPD study of *L. scariosa* var. *novae-angliae* (Kesseli et al., 1998). Conversely, we did not detect strong relationships between population size and quantitative genetic variation, although the number of traits with significant variation in each population declined marginally with decreasing size. Overall, our results suggest that reduced population size may cause a reduction in genetic variation for neutral markers, but not necessarily for quantitative characters, and that neutral marker variation is a poor predictor of quantitative variation. A similar pattern

was found in *Salvia pratensis* (Ouborg and van Treuren, 1994).

Low statistical power could be an obstacle to detecting existing relationships (Podolsky, 2001). Several factors might also decouple the relationship between population size and genetic variation. First, substructuring within populations may enable them to retain variation (Goldstein and Holsinger, 1992; Widen and Andersson, 1993). This possibility gains support from the significant differences among maternal families found for many of the traits, as well as from the substantial within-population substructuring detected in studies of a congener, *L. cylindracea* (Schaal, 1975; Schaal and Levin, 1978). Additionally, long-term population size may exert a stronger influence on both types of genetic variation than current size (Podolsky, 2001), and many of our populations have varied considerably in size in the past century (Kane and Schmitt, 2001). Finally, for quantitative characters, genetic drift can cause random fluctuations in the amount of genetic variation from generation to generation, especially in small populations (Lynch, 1996). Some empirical examinations have found no relationship between population size and either allozyme or quantitative genetic variation (Waldmann and Andersson, 1998; Podolsky, 2001), although another has shown that it is possible for both types of variation to be rapidly lost when effective population size remains small for a number of generations (Briscoe et al., 1992).

In addition, a number of processes may contribute to the decoupling of molecular and quantitative genetic variation (also reviewed by Lynch, 1996; Reed and Frankham, 2001). First, the potential for nonadditive genetic variance to be converted into additive variance after a bottleneck event (Goodnight, 1988; Willis and Orr, 1993) may dissolve the relationship. Second, the higher mutation rates of quantitative characters can allow them to recover variation following a bottleneck or strong purifying selection event more rapidly than single-locus polymorphisms (Lande and Barrowclough, 1987). The ability of bottlenecks to confound relationships among diversity measures has been empirically demonstrated (Cheverud et al., 1994), and the past size fluctuations of our populations indicate that bottlenecks may influence our relationships as well. Third, either stabilizing (Foley, 1992) or directional (Reed and Frankham, 2001) selection on quantitative traits within populations may influence the association between quantitative genetic and allozyme variation. Fourth, the influence of maternal environmental effects may obscure the relationship between variation measures (Reed and Frankham, 2001). Several other studies have also found no relationship between molecular and quantitative genetic diversity (e.g., Cheverud et al., 1994; Waldmann and Andersson, 1998; Podolsky, 2001; Steinger et al., 2002), although one did find an association (Briscoe et al., 1992).

#### 4.4. Conclusions

The addition of our  $G_{ST}/Q_{ST}$  results to those of two similar rare plant studies suggests that low  $Q_{ST}$  may not be infrequent in threatened taxa. We thus reiterate the caution of Petit et al. (2001) that it is misleading to assume that  $G_{ST}$  will always underestimate  $Q_{ST}$  (Lynch et al., 1999). With regard to *L. scariosa* var. *novae-angliae*, the data suggest that the risk of outbreeding depression via the ‘ecological mechanism’ will be low, assuming that this pattern holds for adult traits like phenology. In addition, the  $G_{ST}/Q_{ST}$  technique allowed us to isolate a trait (leaf shape) which did appear locally adapted. Transplanting individuals among populations most similar for this trait, as illustrated in Supplemental Figure 1, would minimize maladapted leaf forms. However, the marker data also suggested restricted gene flow between populations. This means that the potential for outbreeding depression via the ‘genetic mechanism’ cannot be ruled out, and that further experiments are warranted to explore this possibility.

Despite the potential drawbacks, if it were desirable to plan a translocation with only these data, the allozyme genetic distance tree presented in Fig. 2 would be an additional useful tool. Studies in numerous taxa have confirmed the relative generality of a relationship between marker genetic distance and hybrid breakdown (reviewed by Edmands, 2002), although this becomes less reliable at low levels of divergence (Johnson, 2000). Also, recent simulation study indicates that the population structure of loci underlying quantitative traits (=long-term evolutionary potential) should be similar to that for single-locus markers (Latta, 1998; McKay and Latta, 2002). Finally, we would recommend using empirical information (e.g., Supp Tables 4 and 5) to assess the level of genetic variation present in potential donors, as our data suggest that current population size is a poor proxy for these measures.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.biocon.2005.01.021.

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