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# Local adaptation across a climatic gradient despite small effective population size in the rare sapphire rockcress

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When assigning conservation priorities in endangered species, two common management strategies seek to protect remnant populations that (i) are the most genetically divergent or (ii) possess the highest diversity at neutral genetic markers. These two approaches assume that variation in molecular markers reflects variation in ecologically important traits and ignore the possibility of local adaptation among populations that show little divergence or variation at marker loci. Using common garden experiments, we demonstrate that populations of the rare endemic plant *Arabis fecunda* are physiologically adapted to the local microclimate. Local adaptation occurs despite (i) the absence of divergence at almost all marker loci and (ii) very small effective population sizes, as evidenced by extremely low levels of allozyme and DNA sequence polymorphism. Our results provide empirical evidence that setting conservation priorities based exclusively on molecular marker diversity may lead to the loss of locally adapted populations.

**Keywords:** local adaptation; parallel evolution; conservation genetics; drought stress; evolutionary potential; evolutionarily significant unit

## 1. INTRODUCTION

The measurement of genetic variation has become a major focus of conservation research, since many threatened and endangered species are likely to be at risk from genetic as well as demographic determinants of extinction (Lande & Shannon 1996). Typically, within- and between-population surveys of polymorphism using one or more marker types (allozymes, restriction fragment length polymorphisms (RFLPs), microsatellites and nuclear or organelle DNA sequences) are used to estimate phylogenetic and population genetic parameters (Vane-Wright *et al*. 1991; Vrijenhoek 1994; Moritz *et al*. 1995; Hamrick & Godt 1996). Based on the hope that maximizing genetic marker variation will provide remnant populations with the greatest evolutionary potential and reduce the negative consequences of inbreeding, conservation geneticists have advocated preserving the most divergent populations (Moritz *et al*. 1995) or those possessing the greatest level of genetic variation or heterozygosity (Vrijenhoek 1994). These data are also used for deciding which populations are most suitable as restoration sources and for designating evolutionarily significant units, which may be eligible for protection under the US Endangered Species Act (Haig 1998). Although convenient, the current emphasis on neutral marker diversity at the expense of ecological genetic information may lead to poor management decisions for rare species because molecular markers may not reflect variation in ecologically important traits or adaptation to local environmental conditions (Templeton 1986; Cheverud *et al*. 1994; Milligan *et al*. 1994; Hamrick & Godt 1996; Lynch 1996; Storfer 1996; Parker *et al*. 1999; Crandall *et al*. 2000).

Marker diversity is frequently used to decide which populations are most suitable as translocation or restoration sources (reviewed in Templeton 1986; Haig 1998; Knapp & Rice 1998). For example, based on 'low to moderate' levels of genetic differentiation at four microsatellite loci among the three remaining populations of the kokako (*Callaeas cinerea wilsoni*), an endangered passerine bird, Hudson *et al*. (2000) concluded that 'there is no genetic barrier to translocations between the populations' (p. 105). Similarly, based on low levels of genetic variation at randomly amplified polymorphic DNA markers among the four remaining populations of the woody shrub *Grevillea scapigera*, Rossetto *et al*. (1995) concluded that populations were not locally adapted and recommended translocation. However, such management decisions are not without risk, particularly if genetic differences among populations lead to outbreeding depression when local populations are mixed (Fenster & Galloway 2000). In this case, genetic variation in quantitative characters may be more relevant (Geber & Dawson 1993; Schemske *et al*. 1994; Storfer 1996; Waldmann & Andersson 1998; Frankham 1999). Data relating marker diversity to quantitative genetic variation and local adaptation are missing from this debate. Here, we provide a clear demonstration that habitat heterogeneity maintains ecologically important genetic variation among populations of a rare plant even when molecular variation and divergence are largely absent.

## 2. MATERIAL AND METHODS

### (a) *Study organism*

We examined genetic diversity and local adaptation to habitat in populations of the plant *Arabis fecunda* (Brassicaceae), a rare

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Table 1. Microclimatic differences between *A. fecunda* habitats.

(The analysis is based on all data collected from each growing season and the data shown are means at 16.00 in July 1998. Significant differences between means were tested using a two-tailed *t*-test for 1997 and 1998. The soil water potential was not measured in 1997. For details, see § 2(b).)

| habitat differences | low elevation | | high elevation | *p*-value 1997 | *p*-value 1998 |
|---|---|---|---|---|---|
| vapour pressure deficit (kPa) | 2.8 | > | 2.3 | < 0.05 | < 0.005 |
| wind speed ($m\,s^{-1}$) | 2.0 | < | 3.5 | < 0.05 | < 0.0005 |
| air temperature (°C) | 29.5 | > | 24.6 | < 0.0005 | < 0.0005 |
| soil temperature at 15 cm (°C) | 27.2 | > | 24.7 | < 0.0005 | < 0.0005 |
| soil water potential (MPa) | −0.6 | < | −0.46 | — | < 0.0005 |

perennial herb restricted to calcareous soil outcrops in western Montana, USA (Rollins 1993). *Arabis fecunda* is a partially self-fertilizing species (Hamilton & Mitchell-Olds 1994) with 19 existing populations isolated into two elevational groups separated by a distance of 100 km (Lesica 1993). Like many rare plants (Schemske *et al*. 1994), the major threats to this species are human development, competition from invasive exotic plants and cattle grazing (Lesica & Shelly 1996). Of particular concern is a group of four low-elevation (1500 m) populations in the rapidly urbanizing Bitterroot Valley, where active management and reintroduction may be necessary. Differences in phenology driven by snow melt patterns, combined with self-compatibility and geographic isolation, make present-day gene flow between the high- and low-elevation groups of populations extremely unlikely. Hence, divergence by genetic drift is expected.

### (b) *Habitat differences*

We quantified climatic differences between habitat sites for one high-elevation population and one low-elevation population by measuring the air temperature, soil temperature, relative humidity, precipitation, wind speed, global radiation and vapour pressure deficit of the habitats. Weather data were collected simultaneously during the 1997 and 1998 growing seasons using automated weather stations (Campbell Scientific, Logan, UT, USA). We also measured the soil water potential in 1998 using psychrometry (Rundel & Jarrell 1989). These data indicate that plants in the low-elevation habitat were exposed to significantly greater drought stress than those at high elevation in both years (table 1). Because water availability is a fundamental determinant of plant growth and survival (Stebbins 1952; Ingram & Bartels 1996) and dry environments are known to select for higher water use efficiency (WUE) (Dudley 1996), we predicted that differences in water availability would select for local adaptation in drought tolerance, as measured by ecophysiological traits (Comstock & Ehleringer 1992; Geber & Dawson 1993). We tested this prediction and compared the results to between- and within-population differentiation of isozymes and DNA sequences.

### (c) *Molecular markers*

The populations were sampled for marker surveys by collecting seeds from reproductive plants at least 10 m apart. All plant material used for marker analysis was collected from plants grown in a growth chamber from field-collected seeds. In order to assay genetic variation at allozyme loci, we sampled an average of 18 plants per population from eight populations of *A. fecunda* using five out of 15 high-elevation populations and three out of four low-elevation populations. Protein extracts were obtained by grinding 50–100 mg of young leaves in liquid nitrogen and incubating on ice for 10 min with 60 µl of dithiothreitol extraction buffer ($1\,mg\,ml^{-1}$ dithiothreitol in 0.05 M $Na_2HPO_4$, pH 7.0). Isozymes were assayed on 11% starch gel in a morpholine citrate buffer system. Between- and within-population genetic parameters were calculated using GENETIC DATA ANALYSIS (Lewis & Zaykin 2000; see http://lewis.eeb.uconn.edu/lewishome/gda.html).

In order to estimate the effective population size, we measured nucleotide variation at two unlinked nuclear loci. Polymerase chain reaction (PCR) primers for basic chitinase 1 (*Chi1*) and basic chitinase 2 (*Chi2*) were designed using *Arabidopsis* sequences and optimized for *A. fecunda* as described in Bishop *et al*. (2000). All PCR amplifications included water-blank controls. The data for the two loci consists of 67 *Chi1* direct sequences (134 alleles) of 837 bp each from ten populations and 52 *Chi2* direct sequences (104 alleles) of 558 bp each from ten populations. Species and population level $\theta$s (Watterson 1975) were calculated using the number of segregating sites as implemented in DNAsp (Rozas & Rozas 1999). Comparisons to *Arabidopsis* are for coding and non-coding polymorphisms, whereas calculation of $N_e$ from $\theta$ is based only on non-coding regions and synonymous substitutions. We estimated the effective population size from $\theta = 4N_e\mu$, where $N_e$ is the effective population size and $\mu$ is the mutation rate (Kimura & Ohta 1971), by using Koch *et al*.'s (2000) estimate of $\mu = 1.5 \times 10^{-8}\,year^{-1}$ as the neutral mutation rate for relatives of *Arabis* and a generation time of 3 years for *A. fecunda*. The average $N_e$ presented for the species is the mean of the estimated $N_e$ for the 10 populations sampled.

### (d) *Between-population differences in candidate traits*

We examined traits that may confer adaptation to the local climate based on climatic differences between *A. fecunda* habitats. For example, WUE, the carbon isotope ratio and the root mass ratio are traits that may be involved in adaptation to habitat differences during periods of drought (Comstock & Ehleringer 1992; Geber & Dawson 1993). All comparisons among genotypes for each experiment are based on common garden screening in growth chambers or greenhouses. All parameter estimates are based on whole plant measures. In order to estimate within-population variation, full-sib maternal families were produced from two generations of single-seed descent in a common environment in order to reduce maternal effects.

In the first common garden experiment, instantaneous WUE was measured in 42 plants representing three populations from

each elevational group. Each plant came from two generations of single-seed descent of field-collected seed. Seeds were sown directly into 300-ml pots filled with sterile peat potting soils and vermiculite and then cold treated (4 °C) in the dark for 30 days. Seeds were germinated and plants were grown at 25/15 °C and 50/65% relative humidity (day/night) in 15-h days in an environmental growth chamber with fluorescent lighting (photosynthetically active radiation (PAR) = 500 µmol photons $m^{-2} s^{-1}$). We measured gas exchange with a LiCor 6400 instrument (LiCor, Lincoln, NE, USA) equipped with a cuvette that was modified in order to permit whole plant measures of gas exchange, hence integrating leaf performance over the whole plant. Individual values were based on the average of 10 repeated measures and instantaneous WUE was calculated from the ratio of photosynthetic assimilation ($A$ in $CO_2$ µmol $m^{-2} s^{-1}$) to transpiration ($E$ in $H_2O$ mmol $m^{-2} s^{-1}$), where WUE = $A/E$.

### (e) *Quantitative genetic analysis*

In this common garden experiment we determined the degree of heritable variation for WUE, rosette morphology and other ecologically important traits within and between both populations (high and low elevation) for which we collected climatic data. Seeds from eight full-sib families from each of the two populations were placed in Petri dishes with filter paper and 2 ml of tap water. After germination, seedlings were transplanted into peat soil in a randomized complete block design. Plants were grown at 25/20 °C and 60/75% relative humidity (day/night) in 17-h days in an environmental growth chamber with fluorescent lighting (PAR = 150 µmol photons $m^{-2} s^{-1}$). Gas exchange was measured as described above in § 2(d). Three months after germination we measured WUE, rosette height, rosette diameter, shoot biomass and total leaf area. Mortality due to transplanting led to inadequate replication in some families. Therefore, in order to avoid statistical artefacts of unbalanced design, we limited the analysis to five families per population ($n$ = 209 plants). Root biomass was measured in a subset of plants ($n$ = 70) in order to determine the root mass ratio (root mass ratio = root biomass/total biomass). WUE, leaf packing (total leaf area/rosette volume) and leaf area were $\log_e$-transformed in order to meet the assumptions of parametric analysis. The effect of family (d.f. = 8) was considered random and nested within population (d.f. = 1), which was considered fixed. Additional random effects of block and the interaction between family and block were significant for the trait WUE and, thus, added to improve the model fit. The significance of all effects was tested with ANOVA using SAS 6.12 procedure general linear model with type III SS (SAS Institute, Inc., 1996), followed by a sequential Bonferroni procedure in order to control for multiple tests (Rice 1989).

### (f) *Comparative physiology*

In order to test for convergent evolution of WUE in a sympatric *Arabis* species, we compared the instantaneous and long-term WUE (using carbon isotope ratios) in *A. fecunda* and *Arabis holboellii* populations from the high- and low-elevation sites. The analysis of between-population differences in carbon isotope ratios was based on six families per population grown under well-watered conditions in a greenhouse at the University of Montana during the natural growing season. Discrimination against $^{13}C$ is greater with more open stomata and, thus, stable carbon isotope ratios can be used for comparing WUE in plants with similar morphology growing in a common environment (Farquhar *et al.* 1989). The analysis of between-population differences in instantaneous WUE was based on growth chamber screening of at least four individuals per population and the growth conditions were as described above in § 2(d).

## 3. RESULTS

### (a) *Molecular markers*

The levels of neutral genetic differentiation, which were assayed as variation at 14 allozyme loci and the two chitinase loci, were extremely low within and between the *A. fecunda* populations. Of the 14 enzyme loci (*HEX*, *DIA*, *IDH*, *G-6-PDH*, *6-PGD*, *ADH*, *SKDH*, *PGM*, *PGI*, *GOT*, *TPI*, *ME*, *ACP* and *EST*), only *PGI* was polymorphic. *PGI* was polymorphic in only four out of eight populations sampled (two high-elevation and two low-elevation populations) and may be subject to balancing selection (Filatova & Charlesworth 1999). The remaining allozyme loci were monomorphic in all eight populations ($n$ = 142 plants) resulting in a total species diversity ($H_T$) of 0.0076. This level of variation in allozymes is very low, even when compared to mean levels for both endemic ($H_T$ = 0.096) and selfing ($H_T$ = 0.124) plant species (Hamrick & Godt 1996). We partitioned *PGI* variation into a between-population–within-elevation group and between-elevational group components by means of a hierarchical ANOVA of allele frequencies of the *PGI* locus (Weir 1996). The percentage of genetic variation between the high- and low-elevation groups (20%) was lower than the percentage of variation contained among populations within the elevational groups (44%). The within-population allozyme diversities ($H_S$) were 0.0195 ($n$ = 23) and 0.0060 ($n$ = 36) for the low- and high-elevation populations, respectively, at the two sites used for quantitative genetic analysis.

In order to estimate the effective size of the *A. fecunda* populations, we sequenced portions of two nuclear genes, i.e. *Chi1* and *Chi2*. Although we sampled over 170 kb of DNA sequence, only six out of 2330 nucleotide sites were polymorphic and only four of 10 populations contained any nucleotide diversity. Based on all sequence data we estimated a total species $\theta$ of 0.00032. Hence, nucleotide polymorphism in *A. fecunda* is more than 30-fold lower than that found for chitinase in the closely related and highly selfing annual *Arabidopsis thaliana* (Kawabe & Miyashita 1999), where the total species $\theta$ = 0.011. We also calculated the effective population size based on a published estimate of the neutral mutation rate for relatives of *Arabis* (see § 2). The average $N_e$ of 145 (95% CI = 0–530) for *A. fecunda* is more than an order of magnitude lower than the mean census population size of 5000 (Lesica 1993).

### (b) *Local adaptation*

Because *A. fecunda* populations show little divergence at most molecular markers and polymorphism is absent in most populations, one might conclude that any population could serve as a transplant source for recovery at any other site. This conclusion is incorrect. We found that climatic differences (table 1) have led to local adaptation between low- (1525 m) and high- (2195 m) elevation populations. In our first common garden experiment, we found substantial genetically based

Table 2. Quantitative genetic analysis of within- and between-population variation in putatively adaptive traits.

(The means for one high-elevation population and one low-elevation population and ANOVA $p$-values for the independent variables family and population are shown. $p$-values marked with an asterisk indicate significant effects after using a sequential Bonferroni procedure. For details, see § 2(e).)

| traits (units) | low elevation | | high elevation | $p$-value | |
|---|---|---|---|---|---|
| | | | | family | population |
| leaf packing ($cm^2\,cm^{-3}$) | 0.031 | < | 0.047 | 0.352 | 0.0037* |
| leaf area ($cm^2$) | 9.720 | > | 4.840 | 0.279 | 0.0081* |
| root mass ratio (mg root dry mass/mg total plant dry mass) | 0.280 | > | 0.240 | 0.020 | 0.0700 |
| instantaneous WUE ($CO_2\,\mu mol\,m^{-2}\,s^{-1}/H_2O\,mmol\,m^{-2}\,s^{-1}$) | 2.530 | > | 2.010 | 0.200 | 0.0090* |
| rosette height (mm) | 16.380 | > | 8.890 | 0.070 | 0.0001* |
| rosette diameter (mm) | 37.460 | > | 19.590 | 0.001* | 0.001* |

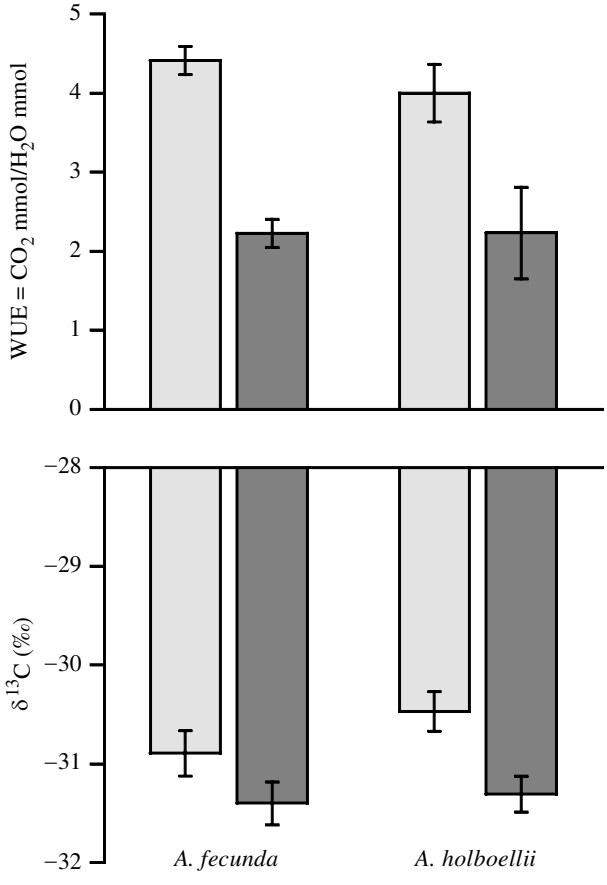


Figure 1. Comparative test of adaptation. Common garden comparison of two sympatric *Arabis* species from the high- and low-elevation habitats. Genetic differences in both (*a*) the instantaneous WUE and (*b*) the stable carbon isotope ratio were tested in independent experiments and indicate genetic differences in stomatal behaviour (Farquhar *et al.* 1989). Differences between low- (light bars) and high-elevation populations (dark bars) are heritable and significant for both species in both traits ($p < 0.005$) (error bars = ± 1 s.e.).

differences in WUE between *A. fecunda* from the two elevation groups. A Mann–Whitney *U*-test showed that the instantaneous WUE for the three populations from the drier, low-elevation habitat, where the mean WUE is 4.38 μmol $CO_2$/mmol $H_2O$, was significantly greater ($p < 0.0001$) than three populations from the wetter, high-elevation sites where the mean WUE is 3.07 μmol $CO_2$/mmol $H_2O$. These genetic differences in WUE between populations may result from physiological differences in gas exchange, as well as morphological differences such as rosette morphology. In our second quantitative genetic experiment, we found significant genetically based differences between populations in other traits affecting growth and physiological performance, further suggesting that *A. fecunda* populations are adapted to their local environments. Table 2 shows that plants from the drier, low-elevation population have greater WUE and larger more open rosettes, as revealed by a quantitative genetic analysis of our common garden study. After correcting for multiple tests, we did not find significant genetic variation in the root mass ratio in the experiment described here, perhaps because the statistical power was reduced by the small sample size for this trait. However, an independent experiment showed that the low-elevation populations have significantly greater root investment (data not shown).

The functional correspondence between genotype, WUE, rosette morphology, biomass allocation and environmental conditions suggests but does not prove adaptive divergence of these traits. Although reciprocal transplants provide a straightforward way of determining whether the genetic differences between populations are adaptive, we felt this approach was not feasible for *A. fecunda*. This is based on concerns of erosion due to the steep, rocky habitat and the danger that reciprocal transplants may cause inadvertent gene flow between populations, resulting in the loss of locally adapted or coadapted genotypes (Fenster & Galloway 2000). Instead, we conducted a comparative test for adaptation, predicting that, if genetic differences in WUE were due to adaptation, then sympatric populations of a congener, *Arabis holboellii*, would show the same pattern of genetic divergence for physiology in response to the gradient in water availability. We tested this prediction in two additional common garden experiments. Figure 1 shows greater WUEs (as estimated by both instantaneous WUEs and stable carbon isotope ratios) in the populations from the drier, low-elevation site for both species. Between-site divergence of WUE for both species combined with

convergent evolution within each site provides further evidence that the between-site differences in WUE are due to local adaptation.

## 4. DISCUSSION

Preserving well-adapted populations is an important goal for species conservation plans. However, within- and between-population studies of marker diversity are routinely substituted for analyses of adaptation to local environments based on the assumption that marker diversity reflects evolutionary potential. In *A. fecunda*, we find that the levels of genetic variation at markers do not predict the levels of variation in quantitative traits, either within or between populations. We found very low levels of genetic variation within *A. fecunda* populations for both traits and molecular markers. The lack of quantitative genetic variation within populations for all but one trait (table 2) is consistent with both the small effective population sizes in this partially selfing species and the expectation of selection on traits involved in local adaptation. Surprisingly, but consistent with results from other taxa (Waldmann & Andersson 1998; Lynch *et al*. 1999), we found no evidence for a correlation between heterozygosity and quantitative genetic variation within populations (data not shown).

Several features of *A. fecunda* may explain the extremely low levels of molecular genetic variation within populations ($H_S$ and $\theta_S$). First, interspecific analyses show that chitinase experiences strong positive selection (Bishop *et al*. 2000), which may reduce sequence variation. However, tests of neutrality show no evidence for selective reduction of variation within species in *A. fecunda* (J. G. Bishop, unpublished data) or *A. thaliana* (Kawabe & Miyashita 1999). Moreover, preliminary comparisons between the two groups of *A. fecunda* populations reveal no sequence polymorphism at *Adh* and only one polymorphic microsatellite out of 22. Thus, it appears that a pattern of low molecular variation is consistent across the *A. fecunda* genome and among molecular marker types. Second, a combination of inbreeding and background selection may drastically reduce the levels of neutral genetic variation in selfing plant populations, where linkage extends across large genomic regions (Charlesworth *et al*. 1997). However, the selfing rate in *A. fecunda* was estimated to be 0.37 (Hamilton & Mitchell-Olds 1994) and therefore does not explain why the total species genetic diversity, $\theta$ and $H_T$, are much lower than that of *A. thaliana* (Bergelson *et al*. 1998; Kawabe & Miyashita 1999) and other highly selfing species (Hamrick & Godt 1996). The near absence of genetic variation within *A. fecunda* may reflect past population bottlenecks resulting from extinction and recolonization events (Pannell & Charlesworth 1999).

The degree of population differentiation in adaptive traits ($Q_{ST}$) under divergent selection pressure is expected to be greater than differentiation in neutral markers ($F_{ST}$) (Rogers 1986; Lande 1992; Prout & Barker 1993; Spitze 1993). In fact the magnitude of the difference between $Q_{ST}$ and $F_{ST}$ can be used for inferring the degree of local adaptation (Prout & Barker 1993; Spitze 1993; Waldmann & Andersson 1998). Comparison of $Q_{ST}$ and $F_{ST}$ in *A. fecunda* also supports our hypothesis of local adaptation: the mean trait divergence of high- and low-elevation populations ($Q_{ST} = 0.94$ and 95% CI = 0.87–1.0) is significantly greater than divergence at the only polymorphic allozyme locus *PGI* ($F_{ST} = 0.20$). Our findings, along with a number of studies finding that the mean $Q_{ST}$ is greater than the mean $F_{ST}$ (reviewed in Lynch *et al*. 1999), are consistent with predictions that population divergence for ecological traits is influenced primarily by natural selection, whereas population divergence for molecular markers is primarily the result of genetic drift. In addition, recent empirical and theoretical results suggest that a small number of loci may explain most of the variation in quantitative traits (Lynch & Walsh 1998; Orr 1998) and, thus, contradict Fisher's (1958) infinitesimal model. If this is generally true, local adaptation will have little influence on measures of overall divergence at neutral markers (Barton & Bengtsson 1986).

A management strategy that uses molecular markers for choosing source populations for transplantation would be misled in *A. fecunda* because adaptation to local environments is not predicted by marker variation. Management of rare species will be more successful if variation in neutral markers is compared to genetic variation in morphological and physiological traits that are expected to confer adaptation to habitat heterogeneity. Applying methods of ecological genetics to conservation can provide direct estimates of the non-neutral genetic variation that imperiled species need to endure current and future abiotic stresses. In addition to reciprocal transplants or alternative methods outlined in this study, Ritland (2000) reviewed how inferences of relatedness derived from molecular data can be combined with field measures of ecological traits in order to estimate levels of quantitative genetic variation, both within (heritability) and between populations ($Q_{ST}$). Finally, the criticism that such ecological genetic measurements are laborious does not justify inappropriate management based on convenient but misleading information.

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