Quantitative Genetic Architecture at Latitudinal Range Boundaries: Reduced Variation but Higher Trait Independence

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ABSTRACT: Species distribution limits are hypothesized to be caused by small population size and limited genetic variation in ecologically relevant traits, but earlier studies have not evaluated genetic variation in multivariate phenotypes. We asked whether populations at the latitudinal edges of the distribution have altered quantitative genetic architecture of ecologically relevant traits compared with mid-latitude populations. We calculated measures of evolutionary potential in nine *Arabidopsis lyrata* populations spanning the latitudinal range of the species in eastern and midwestern North America. Environments at the latitudinal extremes have reduced water availability, and therefore plants were assessed under wet and dry treatments. We estimated genetic variance-covariance (**G**-) matrices for 10 traits related to size, development, and water balance. Populations at southern and northern distribution edges had reduced levels of genetic variation across traits, but their **G**-matrices were more spherical; **G**-matrix orientation was unrelated to latitude. As a consequence, the predicted short-term response to selection was at least as strong in edge populations as in central populations. These results are consistent with genetic drift eroding variation and reducing the effectiveness of correlational selection at distribution margins. We conclude that genetic variation of isolated traits poorly predicts the capacity to evolve in response to multivariate selection and that the response to selection may frequently be greater than expected at species distribution margins because of genetic drift.

Keywords: climatic gradient, evolutionary constraint, **G**-matrix, latitudinal cline, quantitative genetic variation, range margin.

Introduction

Why do species have restricted geographic distributions? There are ecological and evolutionary perspectives on this question. The classic ecological perspective is that distribution limits occur where spatial transition zones in ecological parameters coincide with the boundary of the ecological niche (Brown 1984; Pulliam 2000). Experimental transplants beyond distribution boundaries often observe reduced performance and provide insights into the importance of abiotic and biotic factors in limiting distributions (Hargreaves et al. 2014). The evolutionary perspective is that distribution boundaries reflect limits to niche evolution—a failure of adaptation (Mayr 1963, p. 524). There has been much discussion about genetic mechanisms that account for failure of niche evolution (Bridle and Vines 2007; Kawecki 2008; Sexton et al. 2009) but rather little empirical effort to detect these mechanisms at range margins in nature (Eckert et al. 2008). Here we investigated whether genetic variation of ecologically important quantitative traits and genetic correlations among traits could limit adaptation at the southern and northern boundaries of the range in the brassicaceous plant *Arabidopsis lyrata*.

Theory suggests several factors that may limit adaptation at species' outer range boundaries. Spatially explicit models of distribution along clines find that range limits are caused by a combination of migration from the core and spatial change in the environment (reviewed in Sexton et al. 2009). In Kirkpatrick and Barton's (1997) model of a population that is continuously distributed over a linear cline, adaptation is limited when gene flow from core to edge is strong and the environment changes rapidly. In contrast, quantitative genetics models that assume source-sink dynamics in two discrete habitat patches show that low dispersal can also limit adaptation to marginal conditions (reviewed in Kawecki 2008). In these models, dispersal enhances population size and enables persistence in the sink, and this agrees with the observation that populations at distribution margins tend to occur in marginal habitat, are frequently isolated from gene flow, and have small effective population sizes (Barton 2001; Vucetich and Waite 2003;

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Eckert et al. 2008; Lira-Noriega and Manthey 2014). Under these conditions, the response to selection in edge populations may be constrained by reduced genetic variation and a lower probability of fixation of beneficial genetic variants as genetic drift becomes more important relative to selection (Robertson 1960; Hill and Rasbash 1986*a*, 1986*b*; Wei et al. 1996).

Another possible type of genetic limit to distribution boundaries is caused by correlations among multiple traits due to genetic and developmental trait integration (Antonovics 1976; Arnold 1992; Hoffmann and Blows 1994). Constraint can arise even when all traits have adequate genetic variation when viewed individually, because there may be little or no variation in certain trait combinations (Blows 2007; Walsh and Blows 2009). This multivariate perspective on adaptation employs the genetic variance-covariance (**G**-) matrix to summarize the extent of variation and integration of multiple quantitative traits, predict multivariate evolution, and estimate genetic constraints on evolution (Lande 1979). Simulation models of the evolution of the **G**-matrix illustrate how multivariate constraints could be more severe at distribution boundaries. Jones et al. (2003) found that enhanced genetic drift associated with reduced population size, which is characteristic of demographic conditions at distribution boundaries, causes a reduction in the trace of **G** (sum of the genetic variances) and no change in the multivariate orientation of the matrix. Predictions for the strength of genetic correlations depend on the importance of correlational selection (selection acting on character combinations; Lande and Arnold 1983). Genetic integration is greatly strengthened by correlational selection at large population size but not when drift is important. Thus, the results of Jones et al. (2003) predict that evolutionary potential is reduced in edge populations because of lower genetic variance but may be compensated by weaker trait integration when correlational selection is important.

There is much evidence that populations at range margins have reduced neutral genetic marker diversity (Eckert et al. 2008; Griffin and Willi 2014). However, few studies have mapped the distribution of quantitative genetic variation and genetic integration for ecologically relevant traits to address the causes of distribution limits. For example, Etterson and Shaw (2001) described genetic correlations expected to constrain the response to selection in a prairie legume (*Chamaecrista fasciculata*) along a latitudinal cline, but with only three populations, they could not test for latitudinal trends. Colautti and Barrett (2011) compared the orientation of within- and among-population quantitative genetic variation for 20 populations of *Lythrum salicaria* but did not investigate latitudinal trends in **G**. Calsbeek et al. (2011) compared **G**-matrices for size-related traits in southern and northern populations of a grass (*Phalaris arundinacea*) within the native range in Europe and the introduced range in North America and concluded that south-north differences in **G** were greater than those between introduced and native ranges. These examples offer insight into the evolution of quantitative genetic architecture under population divergence but do not address the limits to adaptation at range edges. What is needed are studies of overall levels of genetic variation and genetic integration in characters that are plausibly related to south-north adaptation and that include populations from the center and geographic edges of the distribution.

Here we describe patterns of quantitative genetic architecture along two parallel latitudinal clines extending to the southern and northern edges of the North American distribution of *A. lyrata* (fig. 1). Even populations a few kilometers apart are highly isolated in this species, indicating strongly limited gene flow (Willi and Määttänen 2010). Populations were sampled over clines that represent gradients in both temperature and water availability (Paccard et al. 2014). Mean temperature during the main growing and flowering season (April to June) ranges from 18°–19°C in the southern-edge populations to 10°–13°C in the northernmost populations; net water availability is reduced by about 40% at both the southern and northern edge regions relative to the center of the distribution. We exposed plants to two watering treatments and measured 10 traits related to water balance and plant size and development. Data on average trait values show that rosette size and the timing of flowering are associated with latitude, with plants from northern locations growing to larger size and flowering earlier (Paccard et al. 2014). In this study, our aim was to determine whether populations at the southern and northern distribution edges differ from central populations in properties of the **G**-matrix that reflect evolutionary potential.

Methods

Sampling Populations

We sampled seeds from nine North American populations of *Arabidopsis lyrata* subsp. *lyrata* in 2011 along two latitudinal clines that extended to the geographic range boundary in both the south and north (fig. 1; table A1, available online). The western cline included populations from Missouri, Iowa, Wisconsin, and western Ontario. The eastern cline included populations from the Appalachians and the East Coast: North Carolina, Virginia, western Maryland, New Jersey, and upstate New York. The two clines fall within separate genetic clusters, based on analysis of microsatellite markers (Willi and Määttänen 2010; Griffin and Willi 2014). From each population, we collected mature fruits from 30 plants

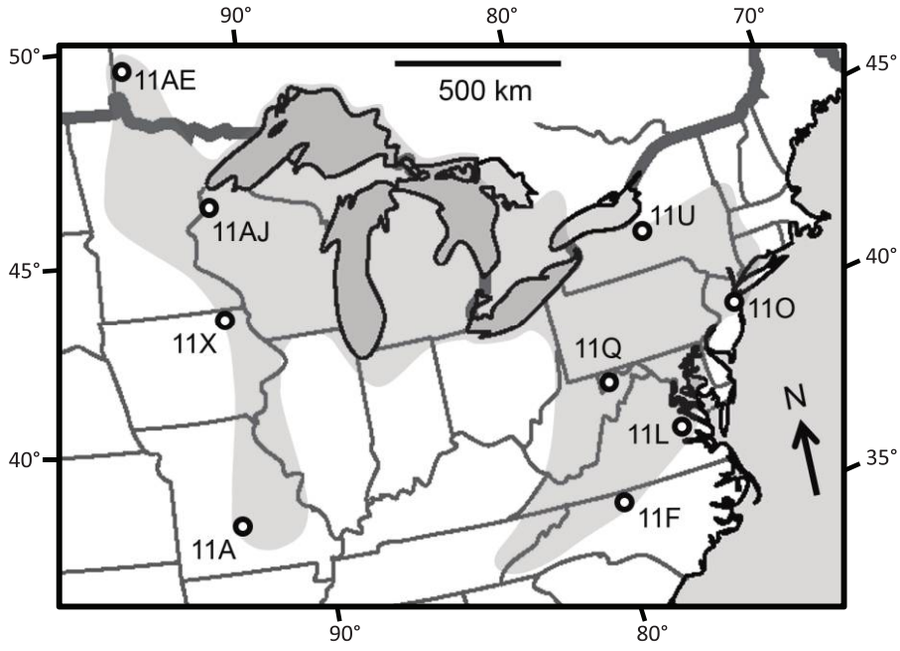

Figure 1: Locations of the nine North American *Arabidopsis lyrata* populations included in this study. The gray shading indicates the distribution of the species based on 591 recent locality records (since 1980) compiled by J. Lee-Yaw and Y. Willi. These records include herbarium entries, regional botanical lists, personal communication with local botanists, and our own field experience. The eastern and western regions represent distinct ancestral genetic clusters (Griffin and Willi 2014).

along with a few backup plants, spaced evenly over a surface area of approximately 500 m^2.

The geographic distribution of *A. lyrata* (fig. 1) does not overlap that of the closely related diploid *Arabidopsis arenicola* to the north or that of the allotetraploid hybrid-species *Arabidopsis kamchatica* to the west (Schmickl et al. 2010). Ecological niche models indicate that the southern and northern distribution boundaries of *A. lyrata* coincide closely with environmental niche limits, whereas the central-western boundary in Iowa and southern Minnesota does not, because suitable habitat occurs farther west (J. Lee-Yaw and Y. Willi, unpublished data). The eastern boundary occurs mainly along the shore of the Atlantic Ocean. Thus, the distribution of *A. lyrata* appears limited by factors associated with latitude, and therefore our study focuses on relationships between quantitative genetic variation and latitude.

Insight into effective population size, mating system, and the fraction of full-sibs among outcrossed offspring comes from analysis of microsatellite data. First, expected heterozygosity (H_E), a proxy for long-term population size in the absence of significant gene flow, is significantly lower at the latitudinal extremes for the nine populations studied here (mean: 0.53; quadratic term ± standard error: -0.0045 ± 0.0018; $P = .043$; data from Griffin and Willi 2014). This implies decreased effective population size at the trailing southern and leading northern edge. Second, the nine populations have a low fixation index ($F_{IS} \leq 0.07$) with no significant latitudinal pattern, indicating that they are all predominantly outcrossing (Willi and Määttänen 2011; Griffin and Willi 2014). Third, a large-scale progeny-array analysis on 13 other outcrossing populations of *A. lyrata* (multilocus outcrossing rate $t_m > 0.8$) revealed that neither the rate of outcrossing nor the fraction of full-sibs (rather than half-sibs) among the outcrossed offspring were linearly or quadratically related to latitude (all $P > .09$; data from Willi and Määttänen 2010). The 13 populations included both central and edge areas in southern and northern parts of the range. The multilocus outcrossing rate and fraction of full-sibs were estimated by genotyping six offspring from each of 30 field-collected seed families per population at 8–10 microsatellite markers ($N = 2,691$ plants in total, including the maternal parents). Inference of shared parentage was based on a "correlated-matings model" with parameters estimated by the program MLTR, version 3.2 (Ritland 2002). The rate of outcrossing averaged 0.925 (standard deviation [SD]: 0.054; range: 0.827–1.030). The estimated fraction of full-sibs averaged 0.166 (SD: 0.057; range:

0.049–0.285). Neither parameter was associated with latitude, so we hereafter disregard the reproductive system and treat all populations as equal.

Experimental Design and Raising Plants

The experimental design consisted of nine populations assessed under two watering treatments, with 30 families per population. There were three replicates (blocks) of every combination of treatment-population-family. This gave 9 populations × 30 families (plus 2 families to compensate for others with few seeds) × 2 treatments × 3 replicates. Ten additional families from each of one southern and one northern population were raised for a separate experiment to estimate natural selection: 2 populations × 10 families × 2 treatments × 2 replicates (see "Drought Selection Experiment" in the appendix; appendix available online). Counting 96 missing plants, the total came to 1,604 plants. In each block, plants were assigned at random to multipot trays and positions within trays. The trays were filled with a 1:1 mixture of sand and peat, and three seeds were sown in small depressions in the soil of each pot (4.4 cm diameter and 63 cm^3). Trays were then placed in a dark chamber at 4°C for 10 days of stratification and sprayed regularly with tap water.

The experiment took place in three growth chambers (CLF Plant Climatics, Wertingen, Germany) with three shelves in each. A particular level (shelf) across the three growth chambers corresponded to one block. At weekly intervals throughout the experiment, we randomly reallocated blocks to a new level and trays to a new position within blocks. The experiment included four phases: germination (day 0 [end of stratification] until day 25), treatment (days 31–73), leaf trait assessment (days 67–76), and flowering time (FT) assessment (day 61 [day of first flower opening] until day 213). Germination conditions were set to 8L : 16D at 18°C with a relative humidity of 40%–60%. Light levels were 150 μmol m^{-2} s^{-1}. We kept the soil wet and recorded germination three times per week. After 17 days, by which time most seeds had germinated, we changed settings in two time steps to longer-day conditions, 14L : 10D at 20°C and 18°C, respectively, and a light level of 200–250 μmol m^{-2} s^{-1}. At the same time, we removed all excess seedlings to leave one plant per pot, chosen at random; 48 excess plants were transplanted into pots with no germinated seedling.

Treatment began when seedlings were well established. Initially, plants in the wet treatment were watered from the bottom twice a week with 1 L tap water per tray. Plants in the dry treatment were watered with 1 L per tray once per week. After 3 weeks, the severity of drought was heightened by increasing the daytime temperature in both treatments to 22°C and providing 0.3 L of water per tray every other day in the dry treatment, applied directly to the individual plants. We measured volumetric moisture content during the first week of the treatment and confirmed that it was less than 10% for the dry treatment and greater than 30% in the wet treatment. In the sand dunes and rocky outcrops inhabited by *A. lyrata* in nature, soil moisture content approaches 0% during dry periods (Y. Willi, personal observation).

Measuring Traits

We measured 10 traits that reflect plant performance or are likely to influence the ability to tolerate dry conditions or extreme temperatures. Growth rate and final size were measured from photographs of the trays made once per week for 7 weeks, starting just before treatments began. We measured the length of the two longest rosette leaves of every plant from the photographs. Because a two-parameter logistic growth model was well-supported by the data for most plants, this was used to estimate growth rate (hereafter abbreviated "grow") and asymptotic rosette radius (hereafter abbreviated "size") for every plant (details in Paccard et al. 2014). Rosette radius measured 21 weeks after treatments began averaged 5.4% larger, which suggests that true asymptotic size had been nearly reached after 7 weeks.

Seven traits reflecting the morphology and physiology of leaves were measured when the first plants started bolting. All trays in both treatments received 1 L water in the morning just before we measured leaf traits, to ensure that plants were not wilted. Water use efficiency (WUE) came from the carbon-isotope ratio (Farquhar and Richards 1984), and photosynthetic capacity came from leaf nitrogen content (Ncont; Reich et al. 1997). Fresh leaf material was dried in a lyophiliser for 24 h, ground for 30 s, and analyzed by isotope mass spectrometry. The spectrometer reported the proportional nitrogen content and the carbon isotope ratio (^{13}C:^{12}C) of the sample (R_s) relative to that of the reference (R_{PDB}), $\delta^{13}C = (R_s/R_{PDB} - 1) \times 1{,}000$ [‰] (Farquhar et al. 1989). We corrected WUE for the ambient carbon isotope ratio by subtracting the average $\delta^{13}C$ obtained from eight corn plants that were reared together with the experimental plants (C_4 plants do not discriminate between the two carbon isotopes).

Leaf shape was quantified with a leaf dissection index (defined as perimeter/[$2\sqrt{(\text{area}\,\pi)}$]; Kincaid and Schneider 1983), measured on two leaves per plant using ImageJ (Rasband 2011). Specific leaf area (SLA; area/dry weight) came from disks of 0.55 cm diameter punched from the same two leaves and weighed after 48 h drying at 60°C (to 0.001 mg precision; disks were 0.3 cm diameter for the

smallest leaves). Trichome density (trich) was the total number of trichomes on the two disks divided by their area. We counted and measured stomata from the impressions they left in clear nail polish painted onto the abaxial side of one leaf per plant. The dried polish was photographed at 100× magnification, and the density of stomata (stomD) was counted over an area of 206,822 μm^2 using ImageJ. Stomata size (stomS) was the distance between guard-cell junctions averaged over 10 stomata.

FT was the number of days between germination and the appearance of the first flower. The earliest plants started flowering near the end of the 42-day treatment period, and we continued to check flowering every 2–3 days until the end of the experiment (213 days after the end of stratification). Dates were adjusted for the midpoint between checks, and we estimated FT for those plants that were within a few days of flowering at the end of the experiment. Plants that had not flowered by the end (39.8%) were assigned a fixed FT of 2 months after the end of the experiment.

Statistical Analysis to Reveal Genetic Parameters

All traits were corrected for tray differences, and three traits were log transformed before analysis to improve their distribution (Ncont, SLA, and trich). About 900 data points were available for each combination of population and treatment: 30 families × 3 replicate plants × 10 traits. This sample size should ensure that the sample covariance matrix approximates the true matrix. Under simplifying assumptions, the number of independent individuals, n, should be at least equal to the number of matrix dimensions, p, for a Gaussian distribution of the data and $p \times \log_e(p)$ for a heavy-tailed distribution (Adamczak et al. 2010; Srivastava and Vershynin 2013). Our sample of 30 families exceeds even the more conservative of these recommendations, which is appropriate for this study given the distribution of our data (fig. A2, A3; figs. A1–A3 available online).

G-matrices summarizing variances and covariances between traits were estimated on the family level by restricted maximum likelihood using package lme4 in program R (R Core Team 2013; Bates et al. 2014). For each population-treatment combination, we fit the following mixed-effects model (VanRaden et al. 1990):

$$Y_{ijk} = \mu + F_{jk} + \varepsilon_{ijk}, \quad (1)$$

where Y_{ijk} is an observation for plant i of maternal family j on trait k, the intercept (μ) is a fixed effect, F_{jk} is the random effect of maternal family, and ε_{ijk} is the random residual. Family and residual within-family effects were modeled as random with unconstrained covariance structure. Likelihood ratio tests were used to evaluate the significance of family-level variances and covariances. The significance of family-level covariances was tested by comparing the model with the full **G**-matrix with one having only genetic variances at the level of family (no covariances among traits at the level of family). The significance of family-level variances was evaluated by comparing the model with only genetic variances with one that had neither variances nor covariances at the level of family. Scripts used for estimating the three kinds of matrices are in table A2, deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.3h3h0 (Paccard et al. 2016).

Models for estimating **G**-matrices were fitted using standardized data (mean = 0 and variance = 1); model fit was good (fig. A2, A3). Two univariate measures of evolutionary potential—heritability (H^2) and Houle's (1992) measure of evolvability (I, or genetic variance divided by the square of the trait mean)—were estimated using analysis of variance on centered data (mean = 0). The genetic variance (V_G) was 3.47 times the variance explained by family. Heritability was calculated using mean squares as $H^2 = 3.47 \times t_{HS/FS}$, where $t_{HS/FS}$ is the intraclass correlation for a mixture of full-sibs and half-sibs (Walsh 2003, p. 4). The value of 3.47 was inferred from the progeny array described above. Because most individuals within the same seed family were half-sibs, **G**, H^2, and I are possibly closer to narrow-sense than broad-sense estimates.

Significance of Houle's I and H^2 was assessed from 500 data sets produced by randomly drawing values with replacement from the original data and assigning them to families, separately for each trait and within categories defined by population and treatment. Houle's I and H^2 were estimated as described above from these 500 data sets, giving a null distribution of each measure against which the observed value was compared. We also tested whether Houle's I and H^2 differed in populations at one or both edges of the distribution by fitting models that included treatment (wet, dry), region (west, east), and linear (centered) and quadratic terms for latitude. This analysis was performed on population- and treatment-level averages of H^2 and Houle's I after standardizing (mean = 0, variance = 1) within traits to ensure their equal contribution to the measure of evolutionary potential.

Analysis of **G***-Matrices*

We calculated four measures of multivariate evolutionary potential and **G**-matrix geometry (size, sphericity, and orientation) for each treatment and population. The first was the trace of the **G**—its size, or the sum of genetic variances across all traits. The second measure was the effective

number of dimensions of the **G**-matrix, or the sum of all eigenvalues of **G** divided by the first eigenvalue (eq. 2 in Kirkpatrick 2009). This measure of matrix sphericity ranges from 1 (all genetic variation is aligned in a single dimension) to the number of dimensions of the **G** (genetic correlations are absent, and variance is equally distributed among traits). The effective number of dimensions indicates whether genetic constraints may exist in certain directions, without indicating any specific direction. Third, we calculated the orientation of **G**-matrices relative to a common standard vector, which was the axis of highest population divergence. This direction, called $\boldsymbol{d}_{\max}$, is the dominant eigenvector of the variance-covariance matrix of population means for 10 traits across the nine populations (the so-called **D**-matrix, describing population divergence). For each population, orientation was measured as the absolute value of the angle between $\boldsymbol{d}_{\max}$ and the direction of greatest within-population genetic variation ($\boldsymbol{g}_{\max}$, the dominant eigenvector of the **G**-matrix for the population).

The fourth measure of evolutionary potential was a modification of Cheverud's "random skewers" method (Cheverud 1996; Cheverud and Marroig 2007). We produced 1,000 random selection gradients ($\boldsymbol{\beta}$, scaled to unit length) and predicted the genetic response to each using the multivariate breeder's equation ($\boldsymbol{\Delta z} = \mathbf{G}\boldsymbol{\beta}$; Lande 1979). For each $\boldsymbol{\beta}$, the capacity to respond was defined as the distance between the end point of $\boldsymbol{\beta}$ and the end point of $\boldsymbol{\Delta z}$. This distance, which we call D, summarizes adaptive constraint with respect to a particular selection regime, because it reflects both the scale and rotation of the response. D is large when there is limited genetic variation in the direction of selection or when the response is deflected at a high angle away from the direction of selection. Although the random $\boldsymbol{\beta}$s inevitably include some that are unrealistic from a biological standpoint, our approach summarizes the capacity of the population to adapt in general, when the actual selection regime is unknown. We calculated the average of D across all randomizations and repeated the procedure for each combination of population and treatment.

Significance of the four measures was evaluated using the same resampled data sets as above, from which **G**-matrices for all populations and both treatments were estimated by fitting equation (1). Randomization should produce **G**-matrices with little variation among families and weak covariances between pairs of traits. The trace, effective dimensions, angle between $\boldsymbol{d}_{\max}$ and $\boldsymbol{g}_{\max}$, and average D calculated from these matrices produced the null distributions of each measure. Relationships with latitude were tested by regression, as described above.

We also measured selection imposed by an extended dry treatment in the growth chambers and estimated the response to this specific selection gradient for each population. The results were similar to those for the random skewers method (details in "Drought Selection Experiment" in the appendix).

Results

There was significant genetic variation and covariation among the 10 traits. Models that included random effects for the off-diagonal genetic covariances among traits were strongly supported by the data in all populations and both treatments (likelihood ratio tests, all $P < .0001$). The same was true for genetic variances except for one population and treatment (11AE in the wet treatment; $P = .107$).

Univariate estimates of Houle's I and heritability were modest in most populations (values reported in table A3, deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.3h3h0; Paccard et al. 2016). The median and interquartile range for Houle's I across traits and populations was 0.007 (0.002–0.019) in the wet treatment and 0.006 (0.002–0.015) in the dry treatment; for H^2, the corresponding values were 0.14 (−0.05 to 0.47) in wet and 0.29 (0.01–0.50) in dry. Average Houle's I tended to be greater than expected by chance (significantly so in two populations in the dry treatment), and average heritability was significantly greater than zero for the majority of populations (table 1). Houle's I averaged across all traits showed a positive and nonlinear relationship with latitude (higher I in the north and somewhat lower at the southern and northern edges of the distribution; table 1; fig. 2*A*). Average heritability was not significantly associated with latitude (fig. 2*B*). Neither Houle's I nor H^2 differed significantly between treatments or regions, and none of the individual traits showed significant relationships with latitude or the square of latitude (all $P > .1$ except once $P = .08$).

The sum of genetic variances (trace of the **G**) tended to be greater than expected by chance in most populations but was significant in only one population-treatment combination (table 1; **G**-matrices for all population-treatment combinations reported in table A4, deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.3h3h0; Paccard et al. 2016). The effective number of dimensions in observed **G**-matrices was higher than that in the resampled data for all 18 combinations of population and treatment (significantly so in six cases). The observed angle between the orientation of genetic variation within populations ($\boldsymbol{g}_{\max}$) and a standard dimension (the among-population variation, $\boldsymbol{d}_{\max}$) was significantly different from expected in four population-treatment combinations; three of these were smaller than expected, and one was larger than expected. The average distance, D, between random selection gradients and the response to selection was smaller than expected by chance in 13 cases (significantly so in three; table 1). In sum, the observed **G**-matrices were somewhat

Table 1: Geographic variation in measures of evolutionary potential in nine populations of *Arabidopsis lyrata*

	No. different from expected ($P < .2$; $P < .05$)		Coefficients with P values in parentheses			
Measure	Dry	Wet	Region	Treatment	Latitude	Latitude^2
Houle's I	2; 2	2; 0	−.082 (.5611)	.221 (.0645)	**.055 (.0083)**	**−.008 (.0310)**
Heritability, H^2	9; 6	7; 5	.006 (.9700)	−.130 (.3350)	.017 (.4258)	−.006 (.1482)
Trace of the **G**-matrix	4; 1	2; 0	.169 (.8704)	−.390 (.6387)	.153 (.2607)	**−.070 (.0148)**
Effective no. of dimensions	5; 3	8; 3	.055 (.6189)	.142 (.1252)	.014 (.3313)	**.008 (.0159)**
Angle between $\boldsymbol{d}_{\max}$ and $\boldsymbol{g}_{\max}$	7; 3	2; 1	−14.07 (.2534)	−8.781 (.3671)	−2.046 (.1974)	.247 (.4111)
Distance between $\boldsymbol{\beta}_{\text{rand}}$ and $\boldsymbol{\Delta z}$	6; 1	3; 2	−.098 (.6231)	−.093 (.5608)	.021 (.4084)	−.008 (.1151)

Note: For Houle's I and H^2, relationships with latitude were tested on average values weighted equally across traits. Trace of **G** is the sum of genetic variances across traits. Effective number of dimensions indicates the sphericity of **G**. The angle between the dominant axis of among-population divergence ($\boldsymbol{d}_{\max}$) and the dominant axis of within-population genetic variation ($\boldsymbol{g}_{\max}$) indicates the orientation of **G**. The average distance between the end points of 1,000 random selection skewers ($\boldsymbol{\beta}_{\text{rand}}$) and the projected response ($\boldsymbol{\Delta z}$) reflects the ability of the population to respond to selection (fig. 2*E*). For each measure, the first two columns are the number out of nine populations for which the observed value differed from expected based on resampling at two levels of significance. Figure 2 illustrates whether each value was larger or smaller than expected. The last four columns are coefficients from analyses that included latitude (centered) and the square of centered latitude, region (west or east), and treatment (wet or dry). Coefficients are reported for the west region and wet treatment. Boldface highlights effects for which $P < .05$.

larger and more spherical than expected by chance and therefore enabled a somewhat better predicted response to arbitrary selection regimes than expected by chance.

Two of four measures of **G**-matrix geometry exhibited nonlinear relationships with latitude (table 1). The concave downward pattern shown by the trace of the **G** pointed toward reduced genetic variance near the southern and northern boundaries of the distribution (fig. 2*C*). This was similar to the result for Houle's I. Populations at both range boundaries, especially in the north, had **G**-matrices with a higher effective number of dimensions, reflecting reduced matrix eccentricity and generally weaker genetic correlations (fig. 2*D*). The angle between $\boldsymbol{g}_{\max}$ and $\boldsymbol{d}_{\max}$ was unrelated to latitude (fig. 2*E*), suggesting that matrix orientation did not change near the southern or northern range edges. There was also no indication of reduced potential selection response at the range edges; if anything, the average distance between $\boldsymbol{\beta}_{\text{rand}}$ and $\boldsymbol{\Delta z}$ decreased slightly toward the edges (fig. 2*F*). Figure A1 shows similar results for the specific selection gradient associated with drought conditions.

Discussion

There is no general consensus on the causes of species distribution limits. One category of explanation involves the quantity and architecture of genetic variation for ecologically relevant traits (Bridle and Vines 2007; Kawecki 2008). We found that the multivariate configuration of genetic variation at the southern and northern edge of the range differs from that of midlatitude populations. In *Arabidopsis lyrata*, the amount of quantitative genetic variation across many traits was reduced in populations at the distribution edges. This pattern was significant for Houle's I and the sum of genetic variances, but it was not significant for heritability. Genetic correlations among traits were less pronounced in edge than midlatitude populations, especially in the north. The consequence of declining V_G but weaker correlations at the edge was that the predicted short-term response to randomly generated selection gradients was not worse or, if anything, slightly better at the southern and northern range margins. Finally, the orientation of the population-specific **G**-matrices showed no pattern with latitude. These findings contradict expectations from a purely univariate perspective on the phenotype, but we believe that they may turn out to be general and could have important implications for understanding species range limits.

The underlying cause of all our results is probably enhanced genetic drift in latitudinal edge populations; this creates reduced **G**-matrix size with no accompanying change in orientation and reduced eccentricity when correlational selection is important. In many taxa, range-edge populations are relatively small, isolated from gene flow, and exposed to elevated rates of genetic drift (Eckert et al. 2008). This seems to be true in *A. lyrata* as well, because variation at microsatellite markers is eroded near the distribution boundaries (Griffin and Willi 2014). Specifically, for the nine populations studied here, marker data show reduced expected heterozygosity in the south and north (see "Methods"). Quantitative genetic models for single and multiple traits all predict that elevated drift leads to reduced genetic variance (Lynch and Hill 1986; Keightley and Hill 1987; Jones et al. 2003). In simulations of **G**-matrix evolution, small effective population size (N_e) is associated with a reduced trace and little change in orientation (Jones et al. 2003). At the same time,

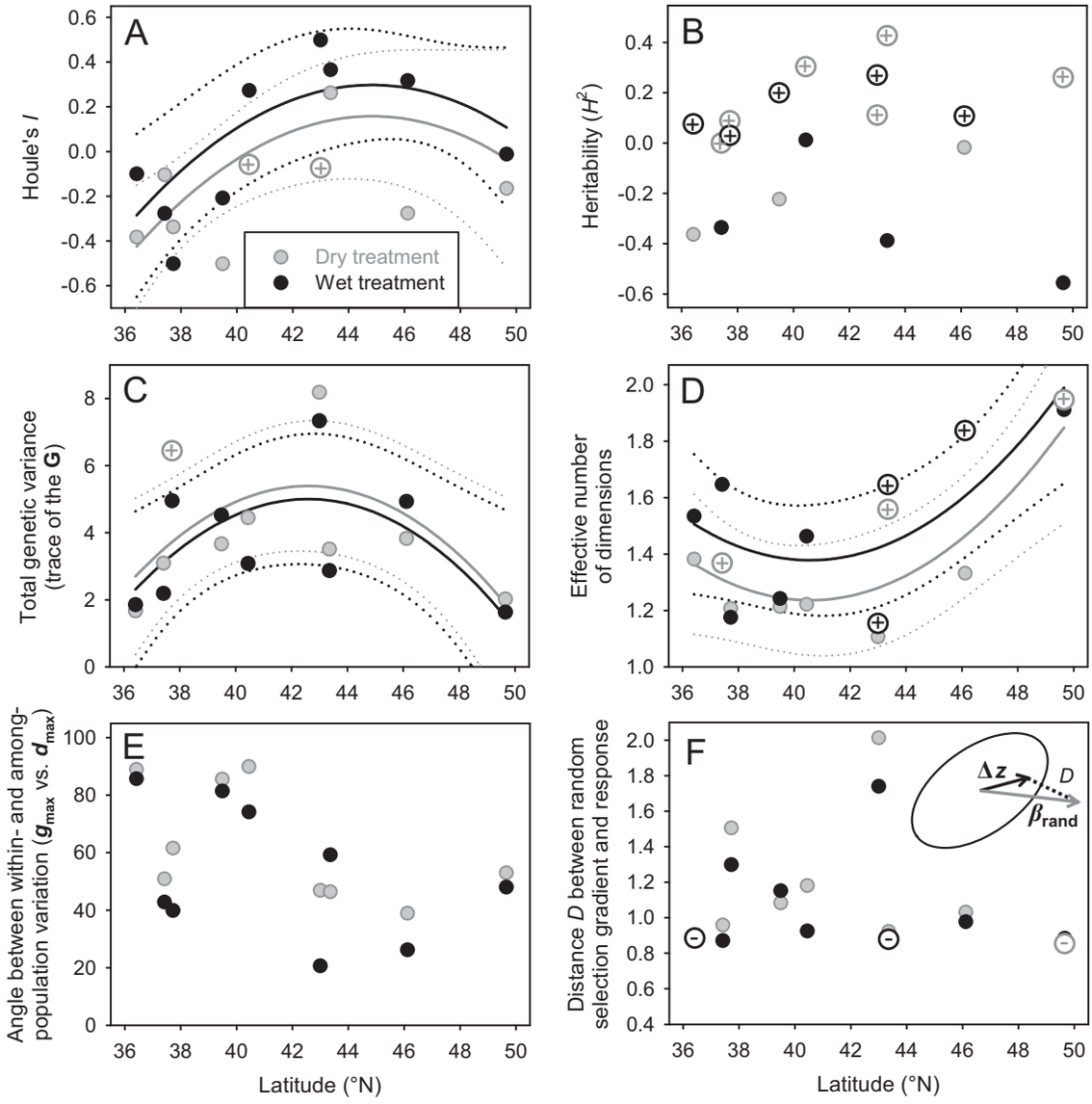

Figure 2: Six measures of genetic variation in nine populations of *Arabidopsis lyrata* sampled across a latitudinal gradient. Each symbol represents one population assessed under wet (black) or dry (gray) conditions. Open symbols were larger (+) or smaller (−) than expected based on a resampling test ($P < .05$). *A* and *B*, Houle's *I* and heritability are mean-scaled and variance-scaled measures of evolvability, normalized by trait and then averaged (see "Methods"). The resampling test was performed on the original estimates. *C*, Trace of the **G**-matrix is the sum of genetic variances across all characters. *D*, Effective number of matrix dimensions is inversely related to the strength of genetic correlations. *E*, Angle between the dominant axis of within-population genetic variation ($\boldsymbol{g}_{max}$) and the dominant axis of population divergence ($\boldsymbol{d}_{max}$) reflects **G**-matrix orientation. *F*, Distance (*D*) between a randomly generated selection gradient ($\boldsymbol{\beta}_{rand}$) and the projected response to selection ($\boldsymbol{\Delta z}$), averaged across 1,000 random gradients, indicates evolutionary potential under selection. The diagram in the corner of *F* illustrates *D* in two dimensions. Solid lines depict linear or quadratic relationships with latitude that were significant; dotted lines are the 95% confidence intervals of the fitted model (table 1).

correlational selection, or selection on combinations of characters, is less effective at maintaining genetic correlations when drift is important. In the simulations of Jones et al. (2003), correlational selection caused a 22% decrease in the effective number of dimensions of the **G**-matrix in a large population ($N_e = 2,731$) but only a 4.2% decrease in effective dimensions in a relatively small population ($N_e = 342$). Although multivariate selection has not been measured in *A. lyrata*, we assume that correlational selection is present, because available evidence indicates that it is nearly ubiqui-

tous (Blows and Brooks 2003). According to this explanation, **G**-matrices at the range margins are more spherical because genetic drift reduces the effectiveness of correlational selection. Figure 2 therefore illustrates the pattern predicted under a model of enhanced drift in range-edge populations: lower V_G and reduced genetic correlations at the southern and northern margins, but no trend in orientation.

Our study is among the first to show reduced genetic variation for quantitative, expressed traits at distribution edges. Most estimates of genetic variation in individual traits find no clear difference between center and edge of the distribution or along range expansion routes. van Heerwaarden et al. (2009) reanalyzed data from three *Drosophila* species along the east coast of Australia, a region representing the entirety, or a good fraction, of the distributions of these species. For two species, no latitudinal trend was found for any of the several traits studied (three and five traits, respectively). For the third species, four traits showed no pattern, whereas two traits showed significant relationships between quantitative genetic variation and latitude in one of 2 years (cold resistance and desiccation resistance). Gould et al. (2014) observed no reduction in quantitative genetic variation from range center to edge for six traits in the annual plant *Clarkia xantiana*. Pujol and Pannell (2008) compared populations in the post-glacial range-expansion portion of the distribution of *Mercurialis annua* to populations within the Pleistocene refugium and found reduced quantitative variation in one of two traits. Our study also recorded no significant reduction in quantitative genetic variation between central and marginal populations when viewing traits individually. It was only when variation was summed across traits that the significant pattern emerged. These results suggest that decreases in quantitative genetic variation may be more salient only when a number of traits are screened, effectively representing a large fraction of the genome.

An important lesson from our study is that patterns of quantitative genetic variance may not reflect the geographic structure of genetic constraint. This is because the response to selection, at least in the short term, is determined by the multidimensional configuration of genetic variation and not by the absolute amount of genetic variance in the population (Walsh and Blows 2009). In spite of reduced quantitative variation in range-edge populations, we find that these populations should respond to natural selection just as effectively as those near the center of the range. This prediction is true for a large number of randomly generated selection gradients (fig. 2*F*) and for the particular selection gradient associated with dry conditions (fig. A1). If anything, the predicted response at the edge of the distribution was slightly better than that at the center. Edge populations of *A. lyrata* were less affected by genetic integration (fig. 2*D*), and this outweighed the constraining influence of reduced total genetic variance (fig. 2*C*). Although our conclusion here agrees with previous studies of single characters that found no decrease in evolutionary potential in range margin populations (van Heerwaarden et al. 2009; Gould et al. 2014), the causal explanation is quite different. This distinction emphasizes the importance of a multivariate perspective on adaptation: univariate analyses of genetic constraint applied to the 10 traits in our study would have suggested a rather different interpretation from multidimensional analyses that account for genetic integration.

Our results, together with those of earlier studies, describe a geographic pattern of population genetic variation consisting of reduced N_e at edges, no consistent pattern in quantitative genetic variation, and no reduced evolutionary potential when genetic integration is accounted for. Does this mean that genetic constraints are not relevant for explaining distribution limits? This conclusion would be premature, we believe, because genetic constraints may be particularly relevant in circumstances that are not represented by existing studies. We describe two examples here. First, most studies include only extant populations, so that we do not witness events in populations beyond the edge of the range where adaptation has failed. Even in edge populations, the environment is sufficiently benign and N_e is sufficiently high to enable persistence and to maintain quantitative genetic variation at levels not much lower than that in core populations. Data from transplants to sites just outside the current range can test the hypothesis that environmental conditions degrade rapidly. Indeed, most (70%) of the transplant studies reviewed by Hargreaves et al. (2014) recorded decreasing fitness beyond current geographical range boundaries, even though transplanted populations were often viable in the short term. Over the long run, if these beyond-range populations were to become established, one imagines that N_e and genetic variance may become critically low. Thus, the range boundary could be enforced by genetic constraints even without any clear pattern in univariate or multivariate quantitative genetic variance in extant populations.

A second circumstance under which genetic constraints would be undetected by existing studies arises because population size influences evolutionary potential independently from its effect on genetic variation. Studies such as ours, van Heerwaarden et al. (2009), and Gould et al. (2014) focus on quantitative genetic variation in range margin populations. But N_e also dictates the rate of accumulation of genetic load, which feeds back to further decrease population size by diminishing the fitness of individuals (Lynch et al. 1995; Whitlock 2000). This positive feedback process, termed "mutational meltdown," should be especially important in the small populations that occur at distribution boundaries and within range expansion zones (Whitlock 2000; Peischl et al. 2015) and is known to re-

duce fitness in small outcrossing populations of *A. lyrata* near the edges of its North American distribution (Willi et al. 2013). There are some self-fertilizing populations at the edges of the species' distribution (Griffin and Willi 2014), and these generally have increased mutational load (Willi 2013), but outcrossing populations predominate even at the edges of the distribution. Mutational meltdown can erode population mean fitness enough to cause extinction directly or can preclude demographic compensation for selective deaths under directional selection (Lynch and Lande 1993; Bürger and Lynch 1995). Thus, small population size can indirectly contribute to a constraint on adaptation at range limits, but this might not be detected by measuring genetic variation.

The hypotheses discussed above are suggested entirely by theory; new empirical data of several types will be required to evaluate which are likely to be important. So far, field studies at the edges of geographic distributions have generated good data on population size, very limited information on genetic variation for expressed traits and accumulation of genetic load, and perhaps no direct information on selection gradients. In addition, experiments investigating the dynamics of genetic variation, drift load, and selection in transplanted populations just beyond the range limit would address the question of why adaptation does not occur there. However, based on available information, our current interpretation of genetic constraint at range limits is that drift is particularly important, potentially causing some erosion of quantitative genetic variation and simultaneously resisting the correlational selection that creates **G**-matrix eccentricity. The result of these two processes is relatively high evolvability at the edge of the range. Additional research will reveal whether this model is general.

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