Weak impact of fine-scale landscape heterogeneity on evolutionary potential in *Arabidopsis lyrata*

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*Arabidopsis lyrata*;
flowering time;
genetic correlations;
G-matrix;
microhabitat adaptation;
quantitative genetic variation;
water-use efficiency.

Abstract

Microhabitat heterogeneity can lead to fine-scale local adaptation when gene flow is restricted, which may be important for the maintenance of genetic variation within populations. This study tested whether microhabitat heterogeneity was associated with trait differences in a population of *Arabidopsis lyrata* and studied its impact on the genetic variance–covariance (G) matrix. Maternal seed families were collected from dune tops and bottoms, two microhabitats known to vary significantly in water availability. In a common garden experiment, replicate individuals per family were raised under wet and dry conditions, and physiological, morphological and life-history traits were assessed. Plants from the two microenvironments differed in their response to treatment in two performance components, in stomata density and most strongly in flowering time. Under wet conditions, plants originating from dune bottoms flowered 4 weeks earlier than those from dune tops. Only one of three G-matrix comparisons revealed that habitat heterogeneity and evolutionary potential were positively linked. The number of independent trait dimensions was larger in the entire population than within subpopulations separated by microhabitat under wet conditions. However, the size of the G-matrix was no larger in the entire population than within subpopulations separated by microhabitat, and trait correlation structure between microhabitats and treatments was not significantly different. These results indicate that fine-scale habitat heterogeneity likely led to local adaptation, which weakly affected levels of across-trait genetic variation.

Introduction

How high levels of genetic variation are maintained in quantitative traits under stabilizing selection remains a major problem in evolutionary biology (Barton & Turelli, 1989; Johnson & Barton, 2005). One explanation arises from three factors acting together: limited gene flow, spatial environmental heterogeneity and selection acting on multiple traits (Barton & Turelli, 1989; Byers, 2005). Environmental heterogeneity can impose divergent selection, even over relatively small spatial scales (e.g. Mojica *et al.*, 2012), which is predicted to maintain genetic diversity directly (Spichtig & Kawecki, 2004; Bürger, 2010) or indirectly via pleiotropy (Barton, 1990). Although theory has produced clear predictions about the maintenance of within-population genetic variation, empirical research in natural populations has been rare. Here, we investigated whether habitat heterogeneity increases multitrait genetic variation by examining trait differences in a plant species distributed across a spatially heterogeneous landscape and comparing genetic variance–covariance (G) matrices between two microenvironments.

Single-locus models show that if dispersal between two habitats is less than a critical value given by the difference in selection between habitats, local adaptation evolves and genetic polymorphism is maintained (Bulmer, 1972; Lenormand, 2002). Quantitative genetic

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models agree that spatial heterogeneity in selection and limited dispersal promote local adaptation (e.g. Kirkpatrick & Barton, 1997), and particularly under soft selection—that is, selection acting locally—these conditions can maintain genetic variation (Spichtig & Kawecki, 2004). The presence of particular genotype-by-environment interactions ($G \times E$) may further help maintain genetic variation (Bürger, 2010). Examples of such $G \times E$ interactions include a correlation of breeding values across habitats of exactly $1$ (Via & Lande, 1985) and the situation in which fitter alleles are partially dominant within each deme, where demes differ in the selection regime (Bürger, 2010).

Mating among nearby individuals is common within populations of many species, and it is in these cases that microhabitat adaptation should occur. This expectation is upheld in herbaceous plants, for which fine-scale genetic structure at neutral loci reflects local mating (Vekemans & Hardy, 2004). Adaptation at a very local spatial scale has been shown to occur in nature, appearing on a scale of around $5$ m in Hydrocotyle bonariensis across a dune landscape (Knight & Miller, 2004), on a scale of $<12$ m in Impatiens capensis (Schmitt & Gamble, 1990) and on a scale of a few hundred metres in Collinsia sparsiflora growing across a mosaic of serpentine and nonserpentine soils (Wright et al., 2006).

Local adaptation may affect more than just the mean and genetic variance of single traits. Selection acts by necessity at the level of the whole phenotype, making evolution a multivariate process (Lande, 1979). Therefore, divergent multivariate selection may maintain genetic diversity in many traits simultaneously. If genetic correlations are strong, selection on one trait may indirectly promote divergence in other, correlated traits (Arnold et al., 2008). Also, because genetic relationships between traits may be strengthened, weakened, or may even change sign depending on the environment in which they are expressed (Falconer & Mackay, 1996; Bégain & Roff, 2001), indirect selection is likely to maintain different patterns of genetic (co-)variance in different environments. Thus, divergent multivariate selection combined with environment-specific trait expression may significantly shape genetic variation in many quantiative traits.

The genetic variance–covariance ($G$) matrix is a convenient way of encapsulating multivariate genetic variation because it depicts both the signature of past adaptive evolution and potential genetic constraints on future evolution (Lande, 1979; Arnold, 1992). Comparisons between closely related populations indicate that $G$-matrices can reflect recent changes in the selection environment. An example is the comparison of $G$-matrices of Brassica rapa collected on a mesic and a dry site before and after a 5-year drought (Franks & Weis, 2008). Traits were assessed under wet and dry conditions, and the pre- and post-drought matrices of the mesic site were found to be similar under wet conditions but share less similarity under experimental water shortage. In contrast, pre- and post-drought matrices of the dry site were very similar under water shortage. $G$-matrix comparisons are commonly used to compare populations whose shared history is not especially recent, but the same method could prove useful for elucidating the effect of microhabitat adaptation on the maintenance of genetic variation within populations.

Here, we examined the effects of divergent selection on the maintenance of genetic variation in a population of Arabidopsis lyrata inhabiting a heterogeneous sand dune landscape on the shore of Lake Michigan, USA. This species is most abundant on unforested or sparsely forested foredunes that provide environmental heterogeneity on the scale of 5–20 m. Dune tops are subjected to strong wind, erosion and sand burial, whereas dune bottoms provide a more stable, sheltered environment. On dune tops, A. lyrata co-occurs with grasses and herbs, whereas the dune-bottom areas have a few trees as well (e.g. Pinus resinosa, P. banksiana). Soil moisture during late spring and summer is considerably lower in open areas than in dune areas with tree stands (Leegte & Murphy, 2001). Plant material for this study was collected from Saugatuck Dunes State Park, Michigan. There, A. lyrata is outcrossing, but spatial autocorrelation analysis has shown that gene flow is limited beyond about 10 m (Willi & Määtätän, 2010, 2011; Appendix S1). Genotype data and density of bolted plants suggested historic mean gene dispersal of 0.8 ± 0.2 m (square root of half the mean squared parent–offspring distance ± SE; SPAGeDi v1.3, Hardy & Vekemans, 2002). Thus, limited gene flow and fine-scale environmental heterogeneity fulfill conditions for the evolution of microhabitat adaptation. Moreover, this population is not strongly influenced by genetic drift: it has high neutral microsatellite gene diversity and a relatively low impact of drift load on population mean performance (Willi & Määtätän, 2011; Willi, 2013; Willi et al., 2013). To test whether microhabitat conditions were associated with trait differences between plants from unforested dune-top areas and plants from forest edge dune-bottom areas, we used a common garden approach with two watering treatments. We then compared $G$-matrices to assess overall changes in the genetic (co)variance structure in response to divergent selection. Specifically, we tested (i) whether pooled families were more genetically diverse than families from either microhabitat alone, (ii) whether pooled families showed more even genetic variation across traits and weaker genetic correlations and (iii) whether families from different microhabitats differed in the structure of genetic variation and covariation. Positive results were interpreted as evidence that microhabitat heterogeneity promoted quantitative genetic variation.
Materials and methods

Sampling and plant rearing

In June 2009, we sampled siliques from plants on unforested dune-top areas (22 plants) and adjacent dune-bottom areas bordering forest (22 plants) at Saugatuck Dunes State Park, Lake Michigan, USA (42°42'N, 86°12'W). Surface area of the sampled region was 5 ha; nearest-neighbour distances among plants averaged 23 m within the same habitat (range: 7–56 m) and 20 m between habitats (4–49 m) (Fig. 1a). One sique was harvested per plant; these potentially contained full- and half-sib seeds.

Siblings were grown under wet and dry conditions. Twelve seeds were haphazardly selected from each maternal seed family and photographed against a white background so that seed size could be measured using ImageJ (Rasband, 2010). Two seeds were planted in each of six pots per family (7 × 7 × 8 cm) in soil containing one part peat and one part sand. Pots of each family were split into six blocks. We stratified seeds at 4 °C for 5 days before removal to the greenhouse in mid-February (average temperature 25 °C, photoperiod increasing from 10 to 12 h). Germination was recorded every 1–3 days. After 1 month, six seedlings per family (one per pot where applicable) were selected and transplanted into newly prepared pots. Individuals from each maternal seed family were randomly assigned to experimental blocks (three levels) and treatments within blocks (two levels). Within blocks, plants from a given treatment were spread over two separate holding trays. Pot position was random within holding tray, and holding trays were randomized weekly within block.

Transplants remained in the greenhouse for a 10-day adjustment period before being subjected to a 12-day vernalization treatment (4 °C, 8 h day) and then moved to indoor culturing facilities (22 °C days, 18 °C nights, 16 h light at approximately 200 μmol m⁻² s⁻¹). We initiated the two treatments, defined by soil volumetric water content (VWC), 1 week after vernalization and maintained them for 4 weeks. Plants in the wet treatment were watered to saturation every 2 days (mean variable water content [VWC] ~30%), whereas plants in the dry treatment were watered on average twice a week (mean VWC < 10%).

Trait measures

We assessed multiple traits thought to be associated with plant performance and coping with drought. Rosette diameter was recorded at weekly intervals during the experiment by measuring the length of the longest line through the centre of each rosette. Rosette size was calculated as the diameter at the end of treatment, when plants had reached an asymptote.

At the end of the treatment period, all plants were given surplus water and leaf traits were assessed. To ensure that the sampled leaves were of the same age, the youngest leaf on each plant was marked with acrylic nail polish prior to treatment. Because A. lyrata produces leaves in a circular pattern of overlaid ‘growth rings’, leaves harvested from above the marked leaf were known to have been produced during the treatment period. We then sampled leaves produced right after treatment began.

We measured carbon isotope ratio (δ¹³C), an integrated measure of water-use efficiency, by collecting 50 mg of fresh leaf material from each plant and immediately drying it for 24 h in a lyophiliser (Edwards freeze dryer Modulyo; Thermo Scientific, Waltham, MA, USA). Once dry, samples were ground for 30 s with a steel bullet in a milling machine (Mixer Mill 300; Retsch, Haan, Germany) and analysed by isotope mass spectrometry at the University of New Hampshire Stable Isotope Laboratory (as per Farquhar & Richards, 1984). The result is the carbon isotope ratio of the probe, Rm relative to the Pee Dee belemnite standard (Rpdbs) (δ¹³C [%o] = (Rm/Rpdbs - 1) × 1000) (Farquhar et al., 1989). Data were corrected for ambient ¹³C:¹²C ratio by subtracting the average δ¹³C value obtained from six corn plants that were raised alongside experimental plants. Because corn utilizes C₄ and not C₃
photosynthetic metabolism, it does not discriminate between the two carbon isotopes and is therefore a suitable reference for the ambient carbon isotope ratio.

Leaf dissection and trichome density were measured on two leaves per plant and averaged before analysis. We photographed the leaves and used ImageJ to estimate leaf perimeter and leaf area. Leaf dissection index (DI) was calculated as DI = perimeter/(2√(area×π)), a parameter without unit and that is 1 for a perfect circle. Trichome density was calculated as the total number of trichomes counted on the adaxial surface of hole-punched discs taken from along the central vein of each leaf, divided by the total disc area (24.15 mm²).

Stomata density per mg dry weight and average stomata length were estimated from one leaf per plant. A small portion was cut from the middle of the leaf, next to the central vein. The abaxial side of the leaf fragment was immediately glued to a microscope slide using liquid adhesive, and the leaf epidermis was removed, leaving the clear cuticle glued to the slide. We photographed cuticle impressions and counted stomata on a surface of 0.207 mm² using ImageJ. Stomata density was expressed relative to a unit of dry leaf mass, measured from area-measured leaves dried for 24 h in an oven at 60°C. Average stomata length was obtained by measuring the distance in micrometres between the guard cell junctions, averaged over eight stomata.

We calculated flowering time (FT) as the number of days between germination and the appearance of the first open flower. FT was assessed every 3 days between germination and the appearance of the first open flower. FT was assessed every 1–3 days between germination and the appearance of the first open flower. FT was assessed every 3 days between germination and the appearance of the first open flower.

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We calculated flowering time (FT) as the number of days between germination and the appearance of the first open flower. FT was assessed every 1–3 days throughout the flowering period. The 21 individuals that did not flower by the end of the experiment, 262 days after planting, were counted as having flowered 20 days later.

Table 1 Multivariate and univariate analyses of variance testing effects of habitat type (H; top and bottom of dunes), treatment (T; wet and dry) and family (Fam) nested within habitat type on seven post-germination traits. N = 240 plants in MANOVA, N = 248–258 for individual traits.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Habitat</th>
<th>Treatment</th>
<th>H × T</th>
<th>Fam (H)</th>
<th>Fam × H × T</th>
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<td>MANOVA</td>
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<td>7.36</td>
<td>162.83***</td>
<td>7.36</td>
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<tr>
<td></td>
<td>7.36</td>
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<td>324,900.46***</td>
<td>1.98***</td>
<td>324,900.46***</td>
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<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Rosette size</td>
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<td>1.28</td>
<td>1006.74</td>
<td>356.15***</td>
<td>9.09</td>
</tr>
<tr>
<td>δ13C</td>
<td>1.38</td>
<td>0.98</td>
<td>235.97</td>
<td>238.95***</td>
<td>0.06</td>
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<td>Leaf dissection</td>
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<td>0.05</td>
<td>0.17</td>
<td>16.43***</td>
<td>0.00</td>
</tr>
<tr>
<td>Trichome density</td>
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<td>0.30</td>
<td>1.00</td>
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<td>0.01</td>
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<td>Stomata density</td>
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<td>0.37</td>
<td>19.11</td>
<td>215.11***</td>
<td>0.41</td>
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<tr>
<td>Stomata length</td>
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<td>0.01</td>
<td>174.06</td>
<td>142.85***</td>
<td>3.15</td>
</tr>
<tr>
<td>Flowering time</td>
<td>1.06</td>
<td>5.03*</td>
<td>0.30</td>
<td>3.01(5)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Habitat was tested over family(habitat) and the effects of treatment and treatment-by-habitat interaction over the treatment-by-family (habitat) interaction. Significance is indicated: *p < 0.1, **p < 0.05, ***p < 0.01, ****p < 0.001.

Statistical analysis

Trait differences between microhabitats and treatments

Genetic differences between habitats and treatment effects were tested using general linear models in SAS (PROC GLM, SAS Institute Inc, 2002). Two traits measured before applying treatments, seed size and mean number of days to germination within a pot, were analysed with a model testing effects of microhabitat (error term: family within habitat) and family nested within microhabitat. All other traits were analysed in a multivariate analysis of variance that included microhabitat of origin, family nested within microhabitat and treatment (error terms reported in Table 1). To improve the distribution of residuals, leaf dissection, trichome density, stomata density and FT were ln-transformed. Days to germination was corrected for block prior to analysis using a linear model, whereas all other traits were corrected for the effects of days to germination and block, separately for the two watering treatments.

G-matrix comparisons

Before genetic variance–covariance matrices were calculated, all traits across the entire data set were standardized to a mean of 0 and a standard deviation of 1 in order to correct for scalar differences. Broad sense G-matrices were estimated for each of the four habitat-by-treatment combinations using the following mixed-effects model:

\[ Y_{ij} = \mu + F_i + e_{ij} \]

where the intercept (\( \mu \)) is a fixed effect and maternal family (F) is a random effect (Dmitriew et al., 2010). Variances and covariances among traits were estimated on the family level by Bayesian analysis with the MCMCglmm package of R v2.15.2 (Hadfield, 2010;
R Development Core Team, 2012; script in Appendix S2) and then multiplied by 2 assuming a full-sib design (Falconer & Mackay, 1996). Total number of iterations was set to 100 000, burn-in to 2000 and thinning to 40. Priors for G-matrices were taken from a mixed model analysis using restricted maximum likelihood (lme4 package of R; Bates et al., 2011) with a moderate degree-of-belief parameter. We used DIC values to evaluate the importance of variances and covariances on the family level in explaining phenotypic variation, in each case comparing models with and without the variance components of interest. Three models were involved: one with the full matrix, one without covariances among traits and one with neither covariances nor variances at the level of family.

We implemented three approaches for comparing G-matrices. The first two compared the G-matrix estimated from the whole population with the two matrices estimated from the separate microhabitats to assess whether microhabitat heterogeneity expanded the dimensionality of genetic variation. This process was repeated for each of the two treatments. First, Bartlett’s test – based on a comparison of the discriminants – was used to determine whether the size of the G-matrix was larger in the whole population compared with the two subpopulations (Goodnight & Schwartz, 1997; Roff et al., 2012). Second, we tested whether the effective number of dimensions of the G-matrix (Kirkpatrick, 2009: eqn. 2) was larger in the whole population compared with the two subpopulations. The effective number of dimensions is the sum of the eigenvalues of G divided by the first eigenvalue. A value of one indicates that all genetic variation is present in a single dimension, whereas higher values indicate that variance is more equally distributed among traits and that genetic correlations between traits are weaker. Finally, in the third approach, we estimated the angles between the G-matrices of the two microhabitats and treatments in a two-dimensional subspace using Krzanowski’s (1979) test. Significance of all comparisons was revealed by randomly resampling individual plants within habitat–treatment combinations, estimating the G-matrices and recalculating test statistics 500 times.

Results

Trait differences between microhabitats and treatments

Seed size did not differ between the two habitat types ($P > 0.8$) but varied significantly among families within habitat ($N = 523, F_{42,479} = 20.04, P < 0.0001$). Similarly, timing of germination did not differ between the two habitat types ($P > 0.6$), but varied among families within habitat type ($N = 263, F_{42,219} = 2.42, P < 0.0001$).

MANOVA on seven post-germination traits revealed a strong overall treatment effect, reflecting substantial phenotypic plasticity in response to drought (Table 1). Under dry conditions, plants were smaller and had higher water-use efficiency, less dissected leaves, more trichomes per leaf surface area, more stomata per dry leaf matter and shorter stomata (Fig. 2). Higher water-use efficiency was revealed by less negative $\delta^{13}C$ values. Only FT did not differ significantly between treatments. There was no consistent habitat effect across treatments, but MANOVA detected a significant interaction between habitat and treatment, caused mostly by FT and stomata density (Table 1). Dune-bottom plants flowered about 4 weeks earlier than those from the dune tops in the wet treatment, but they flowered at the same time in the dry treatment (Figs 1b and 2g). Also, plants from the bottom of dunes had fewer stomata per unit dry matter in the wet treatment (Fig. 2e). There was significant variation among families for all traits except rosette size and no family-by-treatment interactions. Seed size never explained a significant amount of variation when it was included as a covariate in the model ($P \geq 0.16$ for all traits), indicating that maternal effects were probably not important for observed differences between microhabitats.

G-matrix comparisons

Considerable variances and covariances among families were detected among the seven traits (estimates in Appendix S3). For all four habitat–treatment combinations, comparison between a model with no variances–covariances and that with only variances among families (diagonal elements of the G-matrix) supported the importance of broad sense genetic variances (bottom-dry: DIC = 1077.8/1052.2, top-dry: DIC = 960.0/909.5; bottom-wet: DIC = 991.1/985.0; top-wet, DIC = 1083.5/1044.1; a lower DIC value indicates a better model). The comparison between models without and with the off-diagonal elements that represent covariances among traits revealed that covariance terms contributed considerably to model fit (bottom-dry: DIC = 1052.2/1041.1, top-dry: DIC = 909.5/905.0; bottom-wet: DIC = 985.0/958.8; top-wet, DIC = 1044.1/1028.8). The importance of genetic correlations was also indicated by the relatively high amounts of variation explained by the first two components of principal component analyses on the G-matrices (68–74%; Appendix S4).

There was no evidence that habitat heterogeneity expanded the size of the pooled-family G-matrix (Table 2). Bartlett’s statistic was never significantly larger in the total population than in the separate microhabitats. However, habitat heterogeneity increased the effective number of dimensions of the pooled-family G-matrix in the wet but not the dry treatment (Table 2). In the wet treatment, dimensionality was higher in the total population than in the separate microhabitats. This pattern was not driven by an increase in genetic variance in FT or stomata density.
because variances for the two traits were not higher when families were pooled (Appendix S3, S5). Low numbers of effective dimensions (between 2 and 3) confirmed the existence of considerable genetic covariances among traits. Finally, Krzanowski’s comparison of subspaces revealed angles of only 24–33° for subspace 1 and 54–76° for subspace 2, but these values were no different than angles expected at random (Table 2).
Finally, the correlation structure of distributed across traits and/or correlations are weaker in the wet treatment, where FT strongly differed. Pooling families than for the two microhabitats isolation - Bartlett's statistic, of difference in effective number of dimensions, between two G-matrices deviates from random. Significance of Bartlett's test compares the size of the G-matrices between pooled families and habitat (bottom of dunes and top) for the two treatments. Krzanowski's subspace analysis tests whether the angle between the first or second subspace between two G-matrices deviates from random. Significance of Bartlett's statistic, of difference in effective number of dimensions, of subspace angles was revealed by re-sampling: ³P < 0.05.

### Discussion

This study discovered trait differences in a population of *A. lyrata* that were consistent with small-scale microhabitat adaptation to a heterogeneous sand dune landscape. Time from germination to flowering (FT) showed the strongest differentiation between plants derived from open dune tops and forested dune bottoms, but only under wet conditions (Figs 1b and 2g). Stomata density also differed between microhabitats depending on treatment (Fig. 2e). The association between microhabitat heterogeneity and maintenance of genetic variation in quantitative traits was mixed. One measure of genetic variation - the overall 'size' of the genetic covariance (G-) matrix - was no larger when the families from dune tops and bottoms were pooled. However, a second measure of genetic variation - the effective number of dimensions of the G-matrix - was larger for pooled families than for the two microhabitats in isolation in the wet treatment, where FT strongly differed. This means that genetic variation is more equally distributed across traits and/or correlations are weaker (Kirkpatrick, 2009). Finally, the correlation structure of G-matrices did not vary significantly between microhabitats. Thus, habitat heterogeneity and multivariate selection only weakly impacted evolutionary potential and not by simply increasing genetic variances.

Our finding of local differentiation between dune tops and bottoms is unusual in two respects. First, it occurred on a landscape mosaic in which habitat types change within about 20 m. Population divergence and local adaptation are often observed in plants, but the spatial scale is usually regional or geographic rather than local (Leimu & Fischer, 2008). Local adaptation in plants can occur over short distances if selection varies sharply across a distinct microhabitat boundary, such as when plants adapt to heavy metals at mine boundaries, to roadsides, edaphic heterogeneity on serpentine soils or dune position (e.g. Antonovics & Bradshaw, 1970; Wu & Antonovics, 1976; Knight & Miller, 2004; Baythavong et al., 2009). Second, the magnitude of microhabitat divergence in FT - a 31-day difference in the wet treatment - is even larger than that observed in European *A. lyrata* over a latitudinal cline of 14° (Riihimäki & Savolainen, 2004). In this study, the difference amounted to a shift in FT of 0.6 broad sense genetic standard deviations (SD = 52 days).

The common garden experiment demonstrated that plants differ between microhabitats, but did not indicate which environmental features are responsible for divergence. Although the two microhabitats differ substantially in soil moisture (Legee & Murphy, 2001), adaptation in this case may not involve water stress because plants from both habitats were about equally water-use efficient (judging from δ13C) and had similar rosette size. Drought adaptation in herbaceous plants can be accomplished by either early or late flowering, depending on whether the plant escapes or tolerates drought. This is due to a presumably positive genetic correlation between time to flowering and water-use efficiency (Geber & Dawson, 1990; McKay et al., 2003; Juenger et al., 2005; Franks et al., 2007). In our case, plants from the drier dune tops flowered later and had more stomata under wet conditions, but were not more water-use efficient. An additional factor that differs between habitats is canopy cover. Plants from the shadier dune bottoms may experience selection for early flowering, as observed in other species (Donohue et al., 2000). Of course, early flowering in plants is known to be favoured by other environmental factors as well, including high altitude (Hall & Willis, 2006), high latitude (Riihimäki & Savolainen, 2004; Griffith & Watson, 2005) and physical damage (Reisch & Poschlod, 2011).

Habitat heterogeneity in space has been suggested to help maintain genetic diversity within populations (Barton & Turelli, 1989). Experimental evolution studies, mostly with *Drosophila*, have observed either no, a variable or a moderately positive effect of spatial variation on genetic variation (Mackay, 1981; García-Dorado et al., 1991; Yeaman et al., 2010). These experiments...
had good power to detect differences if there were any, which suggests that increases in quantitative genetic variation were not very important. Our approach was quite different – comparing the configuration of G-matrices estimated for the entire population with that estimated in only one habitat type – but the outcome confirmed that the overall quantity of genetic variation was not affected by microhabitat divergence. Flowering time and stomata density differed in mean between top and bottom of dunes under wet conditions, but broad sense genetic variances were not higher when families were pooled. An increase in genetic variances may have been somewhat weakened by assessing plants that were sampled as seeds after gene flow but before selection.

Evolutionary potential depends not only on the quantity of genetic variation. An additional mechanism by which habitat heterogeneity could contribute to maintaining diversity in a population is by altering patterns of covariance among traits. Broad sense G-matrices showed that the seven traits, even though they are associated with quite different functions, were involved in considerable genetic correlations, such that the number of effective trait dimensions was around 2–3 for all habitat–treatment combinations. The effective number of dimensions is strongly influenced by genetic correlations and was larger for pooled families than for the two microhabitats in the wet treatment. Further analyses showed that the orientation of G-matrices compared between habitats was neither more similar nor more different than expected by chance. Depending on the orientation of selection, the different directions of trait correlations in the two microhabitats may slightly facilitate adaptive evolution because of weaker genetic correlations in the whole population.

The structure of G-matrices did not significantly differ between treatments even though the experiment strongly affected most traits. Under drought, A. lyrata plants had higher trichome density, higher density of stomata per dry weight, shorter stomata, higher water-use efficiency (less negative $\delta^{13}$C) and less dissected leaves (Fig. 2). Higher trichome density reduces leaf contact with the air, high stomata density allows for rapid CO₂ diffusion into the leaf, and small stomata close faster; all these lead to less water loss under drought (Hetherington & Woodward, 2003; Picotte et al., 2009). Increased $\delta^{13}$C values result from less selective use of carbon isopotes and reduced stomata opening (Farquhar et al., 1989). Only the smaller leaf dissection indices observed under dry conditions seemed maladaptive, because more pronounced leaf lobes are thought to lower leaf temperature (Nicotra et al., 2011). The relatively small angles between G-matrices in the two treatments, despite large plastic differences between treatments, suggest that multitrait evolvability of the population is not very sensitive to the environment. This agrees with Sherrard et al. (2009), who found that G-matrices based on morphological and performance traits were similar between wet and dry treatments in the plant, Avena barbata.

Our results illustrate that heterogeneous environments together with restricted gene flow can lead to trait differences likely linked with microhabitat adaptation over small spatial scales. The response to heterogeneous selection seems to involve many traits, either as a direct consequence of correlational selection or as an indirect consequence due to genetic correlations. Environmental heterogeneity combines with the multivariate response to selection to somewhat weaken trait integration within the population, without actually increasing genetic variances for individual traits. We suggest that the maintenance of quantitative genetic variation can include properties of the G-matrix and changes in genetic correlations.

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**Supporting information**

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Spatial genetic autocorrelation analysis of the Saugatuck population of *Arabidopsis lyrata* based on 31 samples genotyped at ten microsatellite loci.

**Appendix S2** MCMCglmm code.

**Appendix S3** Genetic variance-covariance matrices of *Arabidopsis lyrata* for the two microhabitats of dune top and dune bottom under the wet treatment (A) and dry treatment (B), estimated with Bayesian analyses and assuming a full-sib design.

**Appendix S4** Eigenvectors of the first two Principal components of a PCA on the G-matrices for each combination of habitat (dune bottom and top) and treatment (wet and dry).

**Appendix S5** Genetic variance-covariance matrices of *Arabidopsis lyrata* based on pooled data of the two microhabitats of dune top and dune bottom, estimated with Bayesian analyses and assuming a full-sib design.

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