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THE EVOLUTION OF QUANTITATIVE TRAITS IN RESPONSE TO DROUGHT IN *ARABIDOPSIS LYRATA*

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Par

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Abstract

In spite of the great advances in population genetic and quantitative genetics over the last decades, many central questions of these fields are still not satisfactorily answered. In particular, we still have a poor understanding of how species are limited in their adaptation to changing environmental conditions and to habitats present beyond their natural distribution. In addition, our knowledge about the effect of habitat heterogeneity on the maintenance of genetic variation remains poor. In the context of global climate changes, many species will have to respond to different environmental conditions in order to survive. Therefore, understanding species' ability to adapt and how high levels of the genetic variance necessary for adaptation can be maintained within populations is highly important. Such knowledge will be very useful for building new conservation strategies.

During this thesis I have investigated these questions using the *Arabidospsis lyrata* plant system. Its ability to grow on different substrates and the development of comprehensive genomic resources makes it a powerful system for studying adaptation. Several seed families occurring in a heterogeneous landscape and across two latitudinal clines in North America were raised in a common garden environment and in two different treatments: wet and dry. By measuring several traits all related to drought adaptation and by performing intense linear and multivariate statics, I discovered that genetic constraints and low levels of genetic variation are limiting northern populations to adapt to higher latitudes. In addition, I observed that habitat heterogeneity did not greatly impact the adaptive potential of this species. Results of this thesis offer a greater understanding of adaptive limits met at distribution edges. This new knowledge will help constructing models evaluating the impact of global changes on many plant and animal populations.

Keywords : adaptation, *Arabidopsis lyrata*, drought, water-use efficiency, G-matrix, latitudinal cline, global changes.

Contents

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Antoine Paccard. March 25th 2013

General introduction

Context and research questions

In spite of the great advances in population genetics and quantitative genetics over the last decades, many central questions of these fields are still not satisfactorily answered. In particular, our understanding of how genetic variation is maintained in populations and how species are limited in their adaptation to changing environmental conditions remains unresolved. In the context of global climate change, it is important to improve our knowledge about the ability of species to adapt, as many conservation strategies will depend on the outcome of such research. This thesis has been devoted to finding answers to mainly three questions. I present them below and provide some information about the theory and past empirical insights.

How is genetic variation for polygenic traits maintained in populations?

After decades of debate, we are still in doubt about the evolutionary processes driving the maintenance of genetic variation within populations. The following processes have been considered important: heterogeneous selection regime and dispersal. While mutation-selection models appear to offer inconsistent results with empirical data (Johnson & Barton, 2005), many models have demonstrated that heterogeneous environments have the potential to create and maintain levels of genetic variance within populations (Gillespie & Turelli, 1989; Bürger & Gimelfarb, 2002; Spichtig & Kawecki, 2004; Turelli & Barton, 2004). In particular, the combination of habitat heterogeneity and low dispersal can sustain levels of genetic variance under local adaptation (Spichtig & Kawecki, 2004). Other processes such as specific genotype-by-environment interactions are also known to maintain genetic variance (Bürger, 2010) but examples of such interactions are still scarce (but see Via & Lande, 1985).

Despite these theoretical advances, empirical work demonstrating the role of environmental heterogeneity on the maintenance of genetic variance remains poor. Several experimental studies on *Drosophila* were performed but revealed conflicting results about the function of environmental variation on genetic variance (Mackay, 1981; García-Dorado *et al.*, 1991; Yeaman *et al.*, 2010). These studies commonly assessed only one or two focal traits for which genetic variances were compared. However, it may be that genetic architecture between traits is important in shaping genetic variance in populations as well (Falconer & Mackay, 1996; Arnold *et al.*, 2008). Therefore, progress may come from the use of the genetic variance-covariance matrix to describe genetic variation in many traits (Arnold, 1992).

Are species adapted to current climatic differences? Are adaptive differences fixed or plastic?

According to theory, long-term adaptation relies on both the actual level of genetic variation and the presence of new mutations (Hill & Rasbash, 1986; Wei *et al.*, 1996). The selection regime and its consistency is another determinant of adaptation to local environmental variables (Robertson, 1960; Grant & Grant, 2002). And, both population size and the different levels of gene flow are known to greatly impact adaptation to habitat conditions (Kawecki & Ebert, 2004; Willi *et al.*, 2006; Willi & Hoffmann, 2009). Basically, if genetic variation is plentiful, and if selection relative to gene flow is strong, then we expect adaptation. Climatic conditions can be assumed important enough to impose divergent selection among populations and to cause divergent climate adaptation, particularly at latitudinal margins of distribution.

At range margins, both fixed adaptive differences and phenotype plasticity can play a significant role in the adaptation of species. Recently, it has been proposed that such adaptation could be facilitated by phenotypic plasticity (Chevin & Lande, 2011). With high gene flow creating maladaptation (Kawecki, 2008; Sexton *et al.*, 2009), strong directional selection would favour plasticity.

We still lack a clear picture of the adaptation patterns along large-scale climatic gradients and the role of adaptive phenotypic plasticity at range margins. In plant species, recent studies have looked at adaptation across altitudes (Hoffmann *et al.*, 2009; Haider *et al.*, 2012) and latitudes (Stinchcombe & Weinig, 2004; Etterson, 2004a) but empirical evidence of co-gradient variation, when trait differences correlate with environmental changes, are still rare (Conover & Schultz, 1995; Johnson & Barton, 2005; Conover *et al.*, 2009). Additionally, few of these studies looked at the extent of phenotypic plasticity along large ecological gradients and particularly at range margins.

Why do species have spatially restricted distributions?

This question, central to the fields of ecology and evolutionary biology, remains open. Several factors can explain limits to adaptation at range margins. The selection regime may be different between core and marginal habitats (Bridle & Vines, 2006; Kawecki, 2008), but strong directional selection is expected at distribution edges (Sexton *et al.*, 2009). As a consequence, both directional selection and small population size will diminish genetic variation and slow down adaptation at range margins (Wright, 1931). Additionally, gene flow may strongly influence adaptation to range margins (Bürger & Lynch, 1995; Sexton *et al.*, 2009). However its effect has been differentially discussed, and models have proposed that gene flow can either promote or limit adaptation (Holt & Gaines, 1992; Lynch & Lande, 1993; Hoffmann & Blows, 1994; Bürger & Lynch, 1995; Kirkpatrick & Barton, 1997; Holt, 2003; Bridle & Vines, 2006).

Extensive progress of theoretical work is based on one- or two-locus models. However, adaptation is more likely to happen in a multivariate fashion, and limits to adaptation in marginal habitats should be investigated by the use of the genetic variance-covariance matrix for deducing evidence for genetic constraints (Lande, 1979; Arnold, 1992). A few studies have chosen this approach. For example, in *Chamaecrista*

fasciculata, Etterson (2004a; b) looked at the evolutionary potential to climate change of three populations growing across latitudes. Comparisons of G-matrices revealed that the northern population might be more constrained in its adaptation. Using a similar method Colautti and Barrett (2008) discovered that the invasive plant *Lythrum salicaria* had been strongly challenged in its invasion to new environments. Despite these results, almost no empirical work looked at the evolution of G across large ecological clines under different conditions and particularly at range margins.

Study system

To answer these general questions I used *Arabidopsis lyrata* subsp l*yrata* as a study system. *A. lyrata* is a close relative of the model species *A. thaliana* and is easy to cultivate and cross-pollinate in the lab. This species has genetically diverse populations and grows over a wide range of habitats (sand dunes, rocky parts, forests). Moreover, *A. lyrata* is an outcrossing diploid and like *A.thaliana* has spatially restricted populations. Finally, this species is found over latitudinal gradients in Europe and North America (Schmickl *et al.*, 2010), suggesting an adaptive potential to the many environmental conditions present across latitudes (e.g. water availability, temperatures, light intensity). Therefore, *Arabidopsis lyrata* is an ideal species for the study of adaptation and evolution of quantitative traits.

This thesis

In this thesis, I tackled these questions, each in a separate chapter. Below is a summary of the approaches taken.

In **Chapter 1**, we investigated whether fine-scale habitat heterogeneity helps maintain genetic variation in ecologically relevant traits. In this study, we used plant material from a heterogeneous sand dune landscape on the eastern side of Lake Michigan, USA. Maternal seed families were harvested from two microhabitats known to differ in their soil water content: the top and bottom of sand dunes. Several individuals per family were raised in a common garden environment under either dry or control/wet conditions. Various traits, all known to be associated with a plant's water balance, were measured and compared across treatment and habitats. Moreover, broad-sense genetic variance-covariance matrices (G) were constructed for all four microhabitat-treatment combinations. Several analyses were carried out to compare G across microhabitats so that the impact of a heterogeneous landscape on the maintenance of genetic variance could be revealed.

In **Chapter 2**, I explored whether populations occurring along two latitudinal gradients in North America displayed trait differences linked to local climatic conditions. Plants were raised under two environmental treatments. I investigated whether differences between treatments were expressed in a fixed or in a plastic way, and whether plasticity was

enhanced at the range margins. Seeds of nine populations were sampled across a range of 13° latitude. Replicate individuals per family were raised under either dry or control/wet conditions for a total of 1620 plants. Several physiological, morphological and leaf history traits were measured. Differences across treatments and populations were revealed by mixed model analysis. Plasticity to drought condition was calculated and differences between central and marginal populations were assessed by linear regression statistics.

Chapter 3 focused on the signature of limits to adaptive evolution at range margins in comparison to centers of distribution. The study was based on the data set of the previous chapter and performed a multivariate statistical analysis to investigate such limitations. Accordingly, I constructed broad-sense G-matrices for each population-treatment combination and compared the ones estimated from populations of range margins with those estimated from populations of the center of distribution. Effects of latitudinal parameters on statistical outputs were revealed by mixed model analysis. Furthermore, comparisons were performed in a pairwise-fashion, and significance was revealed by re-sampling.

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Chapter 1. Fine-scale landscape heterogeneity impacts evolutionary potential in *Arabidopsis lyrata*

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Running title: Microhabitat adaptation in *Arabidopsis*

Abstract

Microhabitat heterogeneity can lead to fine-scale local adaptation when gene flow is highly restricted, and fine-scale adaptation may in turn be important for maintaining genetic variation within populations. This study tested for microhabitat adaptation within a population of *Arabidopsis lyrata* growing across a heterogeneous sand-dune landscape 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, we raised replicate individuals per family under dry and well-watered conditions and assessed physiological, morphological and life history traits. Plants from the two microenvironments differed in their response to treatment in several performance components, most strongly in flowering time. Under well-watered conditions, plants originating from dune bottoms flowered five weeks earlier than those from dune tops. One measure of genetic variation when assessed under control conditions – the number of independent trait dimensions – was larger in the entire population than within subpopulations separated by microhabitat. However genetic variation expressed as 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 can lead to local adaptation, which in turn weakly affects levels of across-trait genetic variation.

Keywords: genetic correlations, quantitative genetic variation, water-use efficiency.

Introduction

Understanding how high levels of genetic variation are maintained in quantitative traits under stabilizing selection remains a major puzzle in evolutionary biology (Barton & Turelli, 1989; Johnson & Barton, 2005). One explanation that is often overlooked 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). Divergent selection may maintain genetic diversity directly (Spichtig & Kawecki, 2004; Bürger, 2010) or indirectly if it affects several traits via pleiotropy (Barton, 1990). We addressed this hypothesis by testing for microhabitat adaptation in a plant species distributed across a spatially heterogeneous landscape and by comparing genetic variance-covariance (G) matrices between two microenvironments.

One-locus models show that if dispersal between two habitat types is smaller 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 models agree that spatial heterogeneity in selection and limited dispersal promote local adaptation (e.g., Kirkpatrick & Barton, 1997). Also, spatial heterogeneity in selection and limited dispersal can maintain genetic variation under soft selection – that is if selection acts locally (Spichtig & Kawecki, 2004). Certain additional conditions, such as the presence of particular genotype-by-environment interactions (G x E), may further help maintain genetic variation (Bürger, 2010). Examples of such G x 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 be especially pronounced. This expectation is upheld in herbaceous plants, which often show fine-scale genetic structure at neutral loci (Vekemans & Hardy, 2004). Adaptation at a very local spatial scale does occur in nature, appearing on a scale of around 5m in *Hydrocotyle bonariensis* across a dune landscape (Knight & Miller, 2004), on a scale of < 12m in *Impatiens capensis* (Schmitt & Gamble, 1990), and on a scale of a few hundred meters in *Collinsia sparsiflora* growing across a mosaic of serpentine and non-serpentine soils (Wright *et al.*, 2006).

Local adaptation may affect more than just the mean and genetic variance of one trait. Selection acts by necessity at the level of the whole phenotype, not on individual traits, making evolution a multivariate process (Lande, 1979). Divergent multivariate selection may therefore maintain genetic diversity in many traits simultaneously, either directly or indirectly. 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; Bégin & Roff, 2001), correlated selection is likely to maintain different patterns of genetic (co-)variance across different environments. Thus divergent multivariate selection combined with environment-specific trait expression may significantly shape genetic variation in quantitative 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 among closely related populations indicate that G-matrices can reflect recent changes in the selection environment. A beautiful example is the comparison of G-matrices of *Brassica rapa* collected on a mesic and a dry site both before and after a five-year drought (Franks & Weis, 2008). The authors assessed traits under control and dry conditions and found that the pre- and post-drought matrices of the mesic site were similar under control conditions but shared little similarity under experimental water shortage. In contrast, pre- and post-drought matrices of the dry site were very similar under water shortage. A classic example where the G-matrix has been used to predict limits to future evolution has been studied in the annual prairie plant *Chamaecrista fasciculata* (Etterson & Shaw, 2001). The authors found that the predicted evolutionary response of the northernmost population to more southern conditions was slower than the predicted rate of climate change, mainly due to the presence of genetic correlations antagonistic to the direction of selection. Commonly, G-matrix comparisons are 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 lyrat*a inhabiting a heterogeneous sand dune landscape on the shore of Lake Michigan, USA. This species is most abundant on un- or weakly forested fore-dunes that provide environmental heterogeneity on the scale

of 5-20m. Dune tops are subject to strong wind, erosion and sand burial, while dune bottoms provide a more stable, sheltered environment. On dune tops, *A. lyrat*a co-occurs predominantly with grasses, whereas the dune-bottom areas have more herbs and a few trees (e.g., *Pinus banksiana*). Soil moisture during late spring is about an order of magnitude lower in open dune-top areas than in dune bottoms with *Pinus* stands (Leege & 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 10m (Willi & Määttänen, 2010, 2011; Appendix S1). Thus limited gene flow and fine-scale environmental heterogeneity provide the 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 little impact of drift load on population mean performance was found (Willi & Määttänen, 2011; Willi, 2013; Willi *et al.*, 2013). To test for local adaptation and phenotypic plasticity in plants from unforested dune-top areas and from forest edges of 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 (1) whether pooled families occupied larger genotypic "trait space" than families from either microhabitat alone, (2) whether the number of effective dimensions of genetic variation – as defined by Kirkpatrick (2009) – was larger in the two habitats pooled than in the sub-populations occurring in the separate microhabitats, and (3) whether families from different microhabitats differed in G-matrix structure.

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). Nearest plants were on average 23m apart within habitat (range: 7-56m) and 20m between habitats (4-49m), over a total surface area of 200m by 250m (Fig. 1A) at Saugatuck Dunes State Park, Lake Michigan, USA (42°42', 86°12'). One silique was harvested per plant; these potentially contained full- and half-sib seeds. In June 2010 we measured volumetric soil moisture content (VMC) at 200 sites including open dune tops and forested dune bottoms on three consecutive days, using a soil moisture meter (Decagon ECH_2O, Pullman, USA; calibrated as per manufacturer's manual). The data are not reported here because they agree with Leege & Murphy's (2001) finding that soil water is lower on open dune tops (e.g., foredunes and blowouts some meters away from pines) than on forested dune bottoms (e.g., under *P. banksiana* at wetpanne edges).

Siblings were grown under well-watered (control) 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 (7x7x8cm) in soil containing one part peat and one part sand. Families were divided equally among six blocks, with pot position randomized within block. Seeds were stratified at 4°C for five days before removal to the greenhouse in mid-February (average temperature 25°C, photoperiod increasing from 10-12h). Throughout the germination period, we watered pots to saturation every 1-2 days and germination was recorded every 2-3 days. A fine mesh

sheet was placed over the pots to maintain humidity around the germinating seeds until >60% germination had been achieved. After one month in the greenhouse, 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 (3 levels) and treatments within blocks (2 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 10d adjustment period before being subjected to a 12d vernalization treatment (4°C, 8h day) and then moved to indoor culturing facilities (22°C days, 18°C nights, 16h light at approximately 200 μmol m^{-2} s^{-1}). We initiated the two treatments, defined by soil moisture content (VWC), one week after vernalization and maintained them for four weeks. Controls were watered to saturation every two days (mean VWC ~30%) while 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 drought tolerance. Rosette diameter was recorded at weekly intervals during the experiment by measuring the length of the longest line through the center of each rosette. Relative growth was calculated by taking the rosette diameter at the end of treatment and dividing it by rosette diameter after one week of treatment.

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. Since *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 that were fully extended and hardened, and at the same position relative to the marked leaf on each plant.

We measured carbon isotope ratio ($\delta^{13}C$), 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, USA). Once dry, samples were ground for 30 seconds with a steel bullet in a milling machine (MM300, Retsch, Germany) and analyzed 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, R_p, relative to the Pee Dee belemnite standard (R_{PDB}) ($\delta^{13}C$ [‰] = (R_p/R_{PDB} - 1) x 1000) (Farquhar *et al.*, 1989). Data were corrected for ambient $\delta^{13}C$ by subtracting the average $\delta^{13}C$ value obtained from six corn plants that were raised alongside experimental plants. Because corn utilizes C_4 and not C_3 photosynthetic metabolism, it does not discriminate between the two carbon isotopes and is therefore a useful reference for the ambient carbon isotope ratio.

Leaf dissection and trichome density were measured on two leaves per plant. We photographed the leaves and used ImageJ to estimate leaf perimeter and leaf area. An index of leaf dissection (DI) was calculated by Fourier transformation, where DI = perimeter / ($2\sqrt{(area*\pi)}$) (Kincaid & Schneider, 1983). DI values are unitless but reach a minimum of 1 for a perfect circle. Trichome density was calculated as the total number of

trichome counted on the upper part of hole-punched discs taken from along the central vein of each leaf, divided by the total disc area (24.15 mm^2). Because leaf dissection and trichome density were assessed on two leaves, we averaged the two measures by plant prior to analysis.

Stomata density per mg dry weight and average stomata length were estimated from one leaf per plant. On each harvested leaf, 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 (Thermo Scientific, USA) using liquid adhesive (Ulrich SA, Switzerland) and the leaf epidermis was removed, leaving 1 μm thickness of clear cuticle glued to the slide. We photographed cuticle impressions and counted stomata on a surface of 0.206 mm^2 using ImageJ. Stomata density was expressed relative to a unit of dry leaf mass, measured from leaf discs dried for 24 h in an oven at 60°C. Average stomata length was obtained by measuring the distance in micrometers between the guard-cell junctions, averaged over eight stomata (Maherali *et al.*, 2002).

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.

Statistical Analysis

Trait differences between microhabitat and treatment. 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 analyzed with a model testing effects of microhabitat (error term: family within habitat) and family nested within microhabitat. All other traits were analyzed 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, relative growth, leaf dissection, trichome density, stomata density and flowering time were ln-transformed. Days-to-germination was corrected for spatial block prior to analysis using general linear models, while all other traits were corrected for the effects of days to germination and block, separately for the two watering treatments.

G-matrix comparison. Before genetic variance-covariance matrices were calculated, all traits 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:

$$Y_{ijk} = \mu + F_i + I_{j(i)} + \varepsilon_{ijk}$$

where the grand mean (μ) is a fixed effect and maternal family (F) and individual (I) nested within family were random effects (Dmitriew *et al.*, 2010). Variance components were estimated by Baysian analysis with the MCMCglmm package of R v2.15.2 (R Development Core Team, 2011; Hadfield, 2010; script in Appendix S2). Total number of iterations was set to 100'000, burn-in to 2'000 and thinning to 40. Priors for G-matrices were taken from a mixed model analysis based on restricted maximum likelihood (lme4 package of R; Bates *et al.*, 2011) with a moderate degree of freedom parameter. We used DIC values to evaluate the importance of broad-sense genetic variances and covariances in explaining phenotypic variation, in each case comparing two models with and without

the variance components of interest. Three models were involved: one with the full G-matrix, one without covariances among traits, and one with neither covariances nor genetic variances at the level of family. MCMC settings were the same as mentioned above.

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. This process was repeated for each of the two treatments. The question was whether microhabitat adaptation expands the dimensionality of genetic variation in the population. First, Bartlett's test – based on a comparison of the discriminants – asked whether the size of the G-matrix was greater in the whole population (Goodnight & Schwartz, 1997; Roff *et al.*, 2012). Second, we used Kirkpatrick's (2009: eqn. 2) measure of the effective number of dimensions of the G-matrix to assess the impact of microhabitat adaptation on multivariate variation and genetic correlations. Finally, we estimated the angles between all four combinations of G-matrices (two microhabitats, two treatments) in a two-dimensional subspace using Krzanowski's (1979) test. Significance of all three comparisons was revealed by randomly re-sampling individual plants, estimating the G-matrices and re-calculating test statistics 500 times. G-matrices of resampled data were calculated based on 30'000 iterations, a burn-in of 1000 and thinning of 25. Priors for G-matrices were taken from a mixed model analysis based on restricted maximum likelihood.

Results

Differences between microhabitats and treatments. Seed size did not differ between the two habitat types ($P > 0.8$) but significantly varied among families within habitat type ($N = 523$, $F_{42,479} = 20.04$, $P < 0.0001$). Similarly, timing of germination did not differ between the two habitat types ($P > 0.7$), but significantly varied among families within habitat type ($N = 263$, $F_{42,219} = 2.66$, $P < 0.0001$). The MANOVA revealed a very strong overall treatment effect, reflecting substantial phenotypic plasticity in response to drought (Table 1). Under dry conditions, plants grew less, had larger $\delta^{13}C$ values, less dissected leaves, more trichome per leaf surface area, more stomata per dry leaf matter and shorter stomata (Fig. 2). The less negative $\delta^{13}C$ values imply less discrimination against $^{13}CO_2$ and therefore higher water-use efficiency. The only trait that did not significantly differ between treatments was flowering time. There was no habitat effect across treatments, but MANOVA detected a significant interaction between habitat and treatment, caused mostly by flowering time (Table 1). Dune-bottom plants flowered about five weeks earlier than those from the dune tops in the control treatment but they flowered at the same time in the dry treatment (Fig. 1B, Fig. 2G). Also, plants from the bottom of dunes had fewer stomata per unit dry matter in the wet treatment (Fig. 2E) and the length of the stomata tended to be shorter under dry conditions (Fig. 2F). Separate analyses also revealed significant variation among families for all traits except growth rate, and no family-by-treatment interactions except for $\delta^{13}C$.

G-matrix comparisons. There were considerable genetic variances and covariances among traits in most of the four habitat-treatment combinations (estimates in Appendix S3). The first component of principal component analyses on the G-matrices

explained 37-46% of the variation; the second principal components explained 24-30% of the variation (Appendix S4). Comparison between a model with no genetic variances-covariances and one with only variances among families (diagonal elements of the G-matrix), respectively, supported the importance of broad-sense genetic variances (bottom-dry: DIC = 1016.7/987.1, top-dry: DIC = 1023.0/981.0; bottom-control: DIC = 1036.2/1000.6; top-control, DIC = 1139.6/1096.2; a lower DIC-value indicates that the model is better at explaining variation). The comparison between models without and with the off-diagonal elements that represent covariances among traits revealed that covariance terms contributed considerably to explain variation (bottom-dry: DIC = 987.1/971.8, top-dry: DIC = 981.0/967.7; bottom-control: DIC = 1000.6/985.6; top-control, DIC = 1096.2/1086.0).

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 dimensionality of the pooled-family G-matrix in the wet but not the dry treatment (Table 2). In the wet treatment, the effective number of dimensions was higher in the total population than in the separate microhabitats. Kirkpatrick's (2009) effective number of dimensions were low (between 2 and 3), confirming the presence of some considerable genetic covariances among traits. Krzanowski's comparison of subspaces revealed angles of only 10-20° for subspace 1, but a much larger 50-80° for subspace 2. These values were neither greater nor smaller than angles expected at random (Table 2).

Discussion

This study discovered small-scale microhabitat adaptation in *Arabidopsis lyrata* in a heterogeneous sand-dune landscape. Time from germination to flowering was the trait that showed the strongest differentiation between plants derived from open dune tops and forested dune bottoms, but only under control conditions (Fig. 1B, Fig. 2G). Stomata density and stomata length also somewhat differed between microhabitats depending on treatment (Fig. 2E, F). Our test of the association between microhabitat adaptation and maintenance of genetic variation in quantitative traits revealed mixed results. A first measure of genetic variation – the overall "size" of the genetic variance-covariance (G-) matrix – did not become 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 the wet treatment, where flowering time strongly differed. Furthermore, the correlation structure of G-matrices did not significantly vary between microhabitats. Thus, habitat heterogeneity and multivariate selection only weakly impacted evolutionary potential, and not by simply increasing variances.

Our finding of local differentiation between dune tops and bottoms is unusual in two respects. First, it occurred over the relatively fine spatial scale of about 20 m. Empirical studies often report population divergence and local adaptation in plants but the scale of comparison 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 micro-habitat divergence in flowering time – a five week difference in the control treatment – is considerably larger than that observed in European *A. lyrata* over a latitudinal cline of 14° (Riihimäki & Savolainen, 2004). In our study this difference amounted to a shift in flowering time of 6 broad-sense-genotypic standard deviations.

The common garden experiment demonstrated that plants differ between microhabitats, but did not demonstrate which environmental features are responsible for divergence. Although the two microhabitats differ substantially in soil moisture (Leege & Murphy, 2001), divergence in this case may not be caused by water stress because plants from both habitats were about equally water-use efficient (judging from $\delta^{13}C$) and had similar growth rates. A more likely factor is canopy cover, which may have imposed selection on plants from the shadier dune bottoms to use lower cue thresholds to initiate flowering under high water availability. Donohue *et al.* (2000) also observed selection for earlier flowering under shaded conditions. Of course other environmental factors that vary over larger spatial scales are also known to favor early flowering in plants, including high altitude (Hall & Willis, 2006), high latitude (Riihimäki & Savolainen, 2004; Griffith & Watson, 2005) and mowing (Reisch & Poschlod, 2011). Curiously, dry conditions can select for either earlier or later flowering, depending on whether the plant tolerates or avoids drought (Geber & Dawson, 1997; McKay *et al.*, 2003; Juenger *et al.*, 2005; Franks *et al.*, 2007).

Our sampling design produced broad-sense estimates of genetic differentiation, which potentially confound genetic differences with maternal effects. In this case,

though, maternal environmental effects were probably small because there was no microhabitat difference in the early-life traits that are commonly associated with maternal effects, such as seed size and timing of germination. In fact, genetic divergence of established plants in the field could be even stronger because the offspring assessed here were the product of some gene flow and had not yet been subjected to selection.

Habitat heterogeneity in space has been suggested to help maintain genetic diversity within populations (Barton & Turelli, 1989) although there is now conflicting evidence from experimental evolution studies. Mackay (1981) investigated the effect of a spatially varying environment and found a moderate positive effect on additive genetic variance in bristle traits and body size of *Drosophila*. Yeaman *et al.* (2010) manipulated the degree of migration in addition to spatial heterogeneity, and found no changes in additive genetic variance or heritability in wing traits and size. García-Dorado *et al.* (1991) manipulated the environmental grain within *Drosophila* cages, and found mixed results in the heritability of sternopleural bristle numbers. 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 from families in only one habitat type – but the outcome confirmed that the overall quantity of genetic variation was not affected by microhabitat divergence.

However, the quantity of genetic variation is not the only consideration; diverse patterns of covariance in different microhabitats is another level at which habitat heterogeneity could contribute to maintaining diversity in a population. Broad sense G-matrices showed that the seven traits, even though they represent quite different

functional aspects, harbored considerable genetic correlations, such that the number of effective trait dimensions was around 2-3 for all habitat-treatment combinations although 7 traits were assessed. The measure of effective number of dimensions of a G-matrix is strongly influenced by genetic correlations, and it was larger for pooled families than for the two microhabitats depending on treatment. A further comparison of the G-matrix structure showed that although angles between subspaces did not deviate from random, they were also not more similar than random. Depending on the orientation of selection within the two microhabitats, the different directions of trait correlations may slightly facilitate adaptive evolution because of more overall diversity in G-matrix structure.

The structure of G-matrices did not significantly differ among treatments even though dry versus wet conditions in the experiment had a strong effect on most of the traits. Subspace analysis revealed a small shared angle between the first subspaces between habitats and treatments. The similarity across habitat type may not surprise because of regular gene flow, but the similarity across treatments is stunning as we found strong plastic responses to treatment for six of the seven traits assessed in directions generally considered adaptive. Under drought, *A. lyrata* plants had higher trichome density, higher density of stomata per dry weight, shorter stomata, higher $\delta^{13}C$ and less dissected leaves (Fig. 2). Higher trichome density reduces leaf contact with the air, high stomata density allows for rapid CO_2 diffusion into the leaf and small stomata can close faster, all leading to less water loss under drought (Hetherington & Woodward, 2003; Picotte *et al.*, 2009). Increased $\delta^{13}C$ values result from the less selective use of carbon isotopes and the minimizing of stomata opening (Farquhar *et al.*, 1989). Only the smaller DI values observed under dry conditions seemed maladaptive as increased lobbing is

thought to lower leaf temperature (Nicotra *et al.*, 2011). The fact that first subspaces had small angles despite large plastic differences between treatments suggests that multi-trait evolvability of the population is to a substantial extent environment-insensitive. In line, G-matrices based on morphological and performance traits of another drought tolerant plant, *Avena barbata*, subjected to the same two treatments showed high similarity (Sherrard *et al.*, 2009).

Our results illustrate that heterogeneous environments combined with restricted gene flow can lead to microhabitat adaptation over small spatial scales. In *A. lyrata*, flowering time plays an important role in microhabitat adaptation. The response to heterogeneous selection seems to be multivariate, either as a direct consequence of selection or as an indirect consequence due to genetic correlations. Our results show that 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 should include properties of the G-matrix and changes in genetic correlations.

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Table 1. Multivariate analysis of variance testing the effect of habitat type (H; top and bottom of dunes), treatment (T; dry and control) and family (Fam) nested within habitat type on relative growth, $\delta^{13}C$, leaf dissection index, trichome density, stomata density, stomata length and flowering time. $N = 240$ plants. Means squares (MS) and F-ratios are reported.

	Habitat		Treatment		H x T		Fam(H)		Fam x H x T	
	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*
MANOVA	$_{7,36}$	1.32	$_{7,36}$	52.45***	$_{7,36}$	2.84*	$_{294,1022.5}$	1.97***	$_{294,1022.5}$	1.06
	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
df	1		1		1		42		42	
Relative growth	0.11	1.97	7.35	107.63***	0.01	0.13	0.06	0.99	0.07	1.22
$\delta^{13}C$	0.23	0.17	192.92	111.48***	0.72	0.42	1.37	1.54*	1.73	1.93**
Leaf dissection	0.02	0.68	0.15	14.50***	0.01	0.96	0.03	2.11***	0.01	0.77
Trichome density	0.02	0.31	0.88	44.95***	0.00	0.21	0.07	2.52***	0.02	0.75
Stomata density	0.13	0.62	18.48	216.49***	0.60	7.01*	0.22	2.35***	0.09	0.93
Stomata length	0.21	0.09	153.37	121.75***	4.74	3.76(*)	2.41	1.61*	1.26	0.84
Flowering time	1.34	6.17*	0.33	2.88	0.86	7.49**	0.22	2.42***	0.12	1.29

The effect of 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: $^{(*)}$ $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 2. Statistics of G-matrix comparison: (A) Bartlett's test, (B) Kirkpatrick's effective number of traits, and (C) Krzanowski's comparison of angles between PCs. Bartlett's test compares the size of the G-matrices between pooled families and habitat (bottom of dunes and tops) for the two treatments (dry and control). Kirkpatrick's effective number of traits is compared between the G-matrices of pooled families and habitat 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.

A. Bartlett's size comparison

dry	bottom, dry	top, dry	control	bottom, C	top, C
	153.32	150.94		69.68	88.58

B. Kirkpatrick's effective number of traits

dry	bottom, dry	top, dry	control	bottom, C	top, C
2.39	2.529	2.185	3.420	2.671*	2.487**

C. Krzanowski's comparison of angles between first two subspaces

	bottom- top, dry		bottom-top, control	
	13.36°	50.33°	20.06°	79.89°
	bottom, dry-control		top, dry-control	
	21.37°	53.59°	14.67°	58.76°

Significance was revealed by re-sampling: * $P < 0.05$, ** $P < 0.01$.

Figure legend

Figure 1. Location of seed families of *Arabidopsis lyrata* from top (open circles) and bottom of dunes (dark circles) collected in Saugatuck Dunes State Park, Michigan, USA in 2009 (A) and their average time to flowering in the control treatment indicated by the symbol size (B). Plants from dune-bottom families started flowering on average about 5 weeks earlier than dune-top families.

Figure 2. Mean of seven performance measures of plants originating from top (open circles) and bottom of dunes (dark circles) under control and dry treatment. Means are based on family means, and data were corrected within watering treatment for germination date and holding-tray prior to graphing. All variables shown are untransformed, while for statistical analysis, relative growth, leaf dissection, trichome density, stomata density and flowering time were ln-transformed.

Figure 1

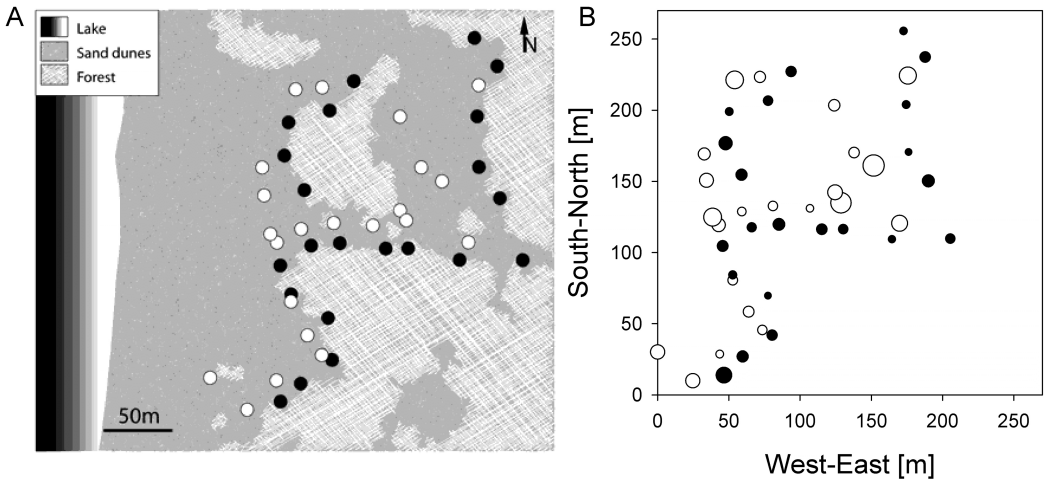
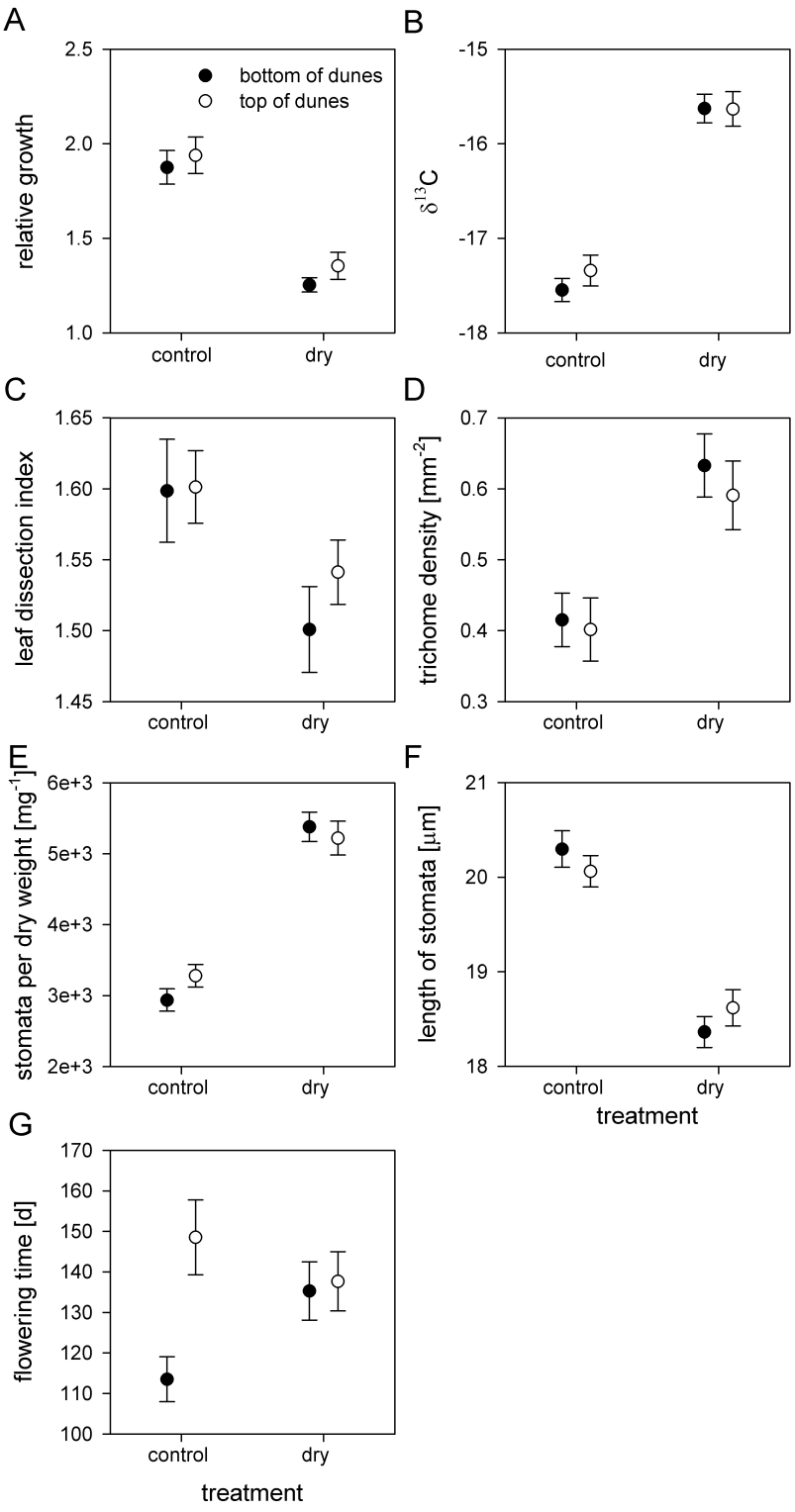
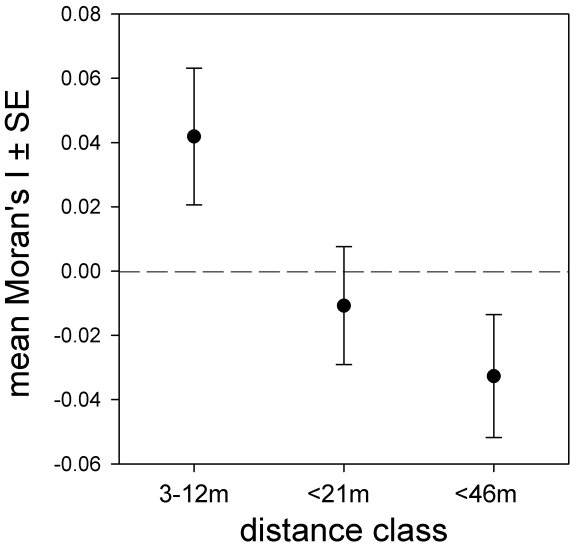

A
Lake
Sand dunes
Forest
N
50m
B
South-North [m]
West-East [m]
0
50
100
150
200
250

Figure 2


A
B
C
D
E
F
G
bottom of dunes
top of dunes
relative growth
δ13C
leaf dissection index
trichome density [mm-2]
stomata per dry weight [mg-1]
length of stomata [µm]
flowering time [d]
control
dry
treatment

Appendix S1. Spatial genetic autocorrelation analysis of the Saugatuck population of *A. lyrata* based 31 samples genotyped at 10 microsatellite loci. Sample sizes for the three distance classes (left to right) were N = 159, 144, 155. Data from Willi & Määttänen (2011).



Appendix S2. MCMCglmm code.

```
m.edgedry <- MCMCglmm(
  fixed = cbind(X1RG, X2DC, X3DI, X4TD, X5SD, X6SL, X7FT) ~ trait - 1,
  random = ~ us(trait):fam,
  rcov = ~ us(trait):units, prior=prior.edgedry, family=c("gaussian", "gaussian",
"gaussian", "gaussian", "gaussian", "gaussian", "gaussian"),
  nitt=100000, burnin=2000, thin=40, data=d.edgeD)
VC.edgedry <- matrix(posterior.mode(m.edgedry$VCV), nrow=Ntraits, ncol=Ntraits)

prior.edgedry <- list(R=list(V=diag(7), n=1),
              G=list(G1=list(V=vc.edgedry.lmer, n=4)))
```

Appendix S3. Genetic variance-covariance matrices of *Arabidopsis lyrata* of the two microhabitats of dune top and dune bottom under the control, well-watered treatment (A) and dry treatment (B). Estimates for dune tops are on the upper right side of the matrices, estimates of dune bottoms are on lower left side of the matrices. The traits were relative growth (RG), $\delta^{13}C$, leaf dissection index (DI), trichome density (TD), stomata density (SD) and length (SL), and flowering time (FT). Traits were standardized to a mean of 0 and a standard deviation of 1 prior to analysis, across habitats and treatments.

A. Control	RG	$\delta^{13}C$	DI	TD	SD	SL	FT
RG	0.426/0.266	-0.180	-0.029	-0.066	-0.039	0.130	0.042
$\delta^{13}C$	-0.152	0.262/0.271	-0.096	-0.049	0.152	-0.164	-0.090
DI	0.089	-0.169	0.475/0.217	-0.080	-0.183	0.037	0.110
TD	-0.156	0.006	0.016	0.182/0.325	0.081	-0.050	0.001
SD	0.016	0.036	0.051	-0.084	0.416/0.257	-0.104	-0.107
SL	0.043	-0.138	0.033	0.051	-0.379	0.328/0.158	0.062
FT	0.012	0.103	0.030	-0.109	0.116	-0.102	0.193/0.681

B. Dry	RG	$\delta^{13}C$	DI	TD	SD	SL	FT
RG	0.212/0.174	-0.202	0.020	-0.117	-0.169	0.118	-0.010
$\delta^{13}C$	-0.196	0.259/0.335	-0.006	-0.015	0.192	-0.165	-0.010
DI	0.028	-0.011	0.256/0.111	-0.080	-0.104	-0.111	0.137
TD	-0.108	0.116	-0.019	0.213/0.363	0.223	-0.057	-0.087
SD	-0.115	0.066	-0.040	0.002	0.182/0.328	-0.091	-0.022
SL	0.085	-0.104	0.108	-0.076	-0.072	0.242/0.293	-0.075
FT	0.013	-0.034	0.079	-0.151	0.079	-0.013	0.306/0.251

Appendix S4. Eigenvectors of the first two principal components of a PCA on the G-matrices estimated by maximum likelihood within each combination of habitat (dune bottom and top) and treatment (dry and control). The bottom line reports % variance explained by each principal component.

	Bottom, dry		Top, dry		Bottom, control		Top, control	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Relative growth	0.485	-0.206	0.413	-0.105	-0.098	0.588	-0.240	-0.397
$\delta^{13}C$	-0.523	0.138	-0.470	0.344	0.284	-0.403	0.381	0.373
Leaf dissection	0.242	0.142	0.100	0.407	-0.102	0.610	-0.296	-0.090
Trichome density	-0.410	-0.322	-0.410	-0.477	-0.149	-0.228	0.116	0.259
Stomata density	-0.233	0.425	-0.562	-0.166	0.646	0.245	0.378	0.256
Stomata length	0.397	-0.185	0.333	-0.464	-0.621	-0.032	-0.270	-0.308
Flowering time	0.230	0.774	0.062	0.484	0.272	0.082	-0.693	0.684
% total variance	0.40	0.25	0.46	0.28	0.37	0.30	0.40	0.24

Chapter 2. Latitudinal variation in response to drought in *Arabidopsis lyrata*

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Running title: Plastic drought responses across latitude

Abstract

Species may respond in one of three ways to environmental change: adapt, migrate or go extinct. Many plant species do not have the ability to physically escape environmental change and their persistence is likely to depend on their potential to adapt. Studies of latitudinal clines can provide information on whether species have been able to adapt to abiotic stress such as drought or temperature extremes in the past, and on conditions that may imply selection limits. In the present study, we investigated whether *Arabidopsis lyrata* subsp. *lyrata* populations have differentially adapted to drought across two latitudinal gradients in North America. Plants from nine populations were grown under dry and well-watered conditions. A total of 1620 seedlings were raised and 8 traits related to the plants' water balance were measured. Most traits displayed a plastic response to drought. Flowering time was significantly associated with latitude while water-use efficiency showed a similar trend; plants from northern locations (with a drier spring season in general) flowered earlier and were to some extend more water-use efficient compared to plants from southern locations, independent of treatment. These observations offered evidence of co-gradient variation. Plastic responses to drought were not enhanced at range margins. We conclude that plants along latitudinal clines are differentially adapted to precipitation patterns. And, contrary to theoretical predictions, evolution at range margins does not seem to involve enhanced phenotypic plasticity.

Keywords: latitudinal cline, phenotypic plasticity, *Arabidopsis lyrata*, global change.

Introduction

Global climate change will have a major impact on species, and it is therefore essential to study their ability to cope with rather rapid habitat modifications (Palumbi, 2001; Parmesan, 2006).When species face environmental change, they can respond in three different ways: adapt, shift their geographic range or go extinct (Bell & Collins, 2008). Many organisms are unlikely to persist by moving their distribution as their ability to disperse is rather restricted (Venable & Brown, 1993). Thus, population persistence of such species depends on their propensity to show an adaptive phenotypic response or to evolve and adapt to environmental change. The ability of species to cope with such change can be studied by looking at the extent of current presumably adaptive trait differences found across climate gradients. Many species grow over North-South gradients and cover a wide range of climatic conditions. Studying evolution over a latitudinal cline can help understand how they have adapted to climatic differences in the past and how they may be limited in their adaptation at range margins. This approach is known as space-for-time-substitution (Pickett, 1989), where current spatial differences in climate are used to predict the traits that are likely to be under selection under future climate change.

Several factors affect the extent to which a species or population can adapt to local climatic conditions. According to quantitative genetics theory, the long-term selection response depends both on the initial genetic variation for the trait under selection and on the appearance of new mutations (Hill & Rasbash, 1986; Wei *et al.*, 1996). Furthermore, the long-term selection response is proportional to the intensity of selection as well as its duration (Robertson, 1960; Hill & Rasbash, 1986). However, in

nature, consistency in the selection regime may be more important in determining the directional selection response (Grant & Grant, 2002). In addition, population size is highly relevant because it positively influences the probability of fixation of new mutations and the predominance of selection over genetic drift (Robertson, 1960; Hill & Rasbash, 1986; Wei *et al.*, 1996). A further factor influencing local adaptation is gene flow. While low gene flow is beneficial to the adaptation process, high rates can swamp local adaptation by maladapted genes, with the consequence that populations never reach their adaptive optimum (Kawecki, 2008). At range margins, while populations may be limited by demographic problems leading to inbreeding and reduced genetic variation, the overall theoretical understanding is that both small population size and gene flow from core habitats are the major causes for a limit to adaptation at a species' range margin (Kawecki, 2008).

Instead of fixed adaptive differences, an alternative evolutionary trajectory at range margins may be one of adaptation by phenotypic plasticity. Phenotypic plasticity means that trait expression is adjusted to environmental cues during development or any later point of life (Scheiner, 1993). Marginal habitats may be more environmentally variable than core habitats, and therefore, selection may act on the plasticity in trait expression (Levins, 1968; Sexton *et al.*, 2009). Chevin & Lande (2011) suggested that gene flow at range margins may generally increase maladaptation, and that continuous directional selection towards the optimum phenotype will favor the evolution of plasticity. As a result, maladaptation would be diminished and fitness increased, leading to larger population sizes and the invasion of new habitats.

Evolved differences in trait expression across clines that are presumably adaptive are best studied in common garden experiments. In a common environment, environmental effects on trait expression are consistent between populations, so that significant differences in traits can be interpreted as genetic differences due to past evolutionary shifts. Moreover, when such experiments include the manipulation of the environment imitating environmental differences in nature, they have the potential to reveal evidence for co- or counter-gradient variation (Conover *et al.*, 2009). Evidence for co-gradient variation is given if genetic trait differences correlate with environmental changes found along the cline. Conversely, counter-gradient variation occurs when genetic and environmental effects on phenotypes oppose each other along the cline. Systematic patterns of these kinds can be interpreted as the result of selection and adaptive evolution instead of genetic drift (Conover & Schultz, 1995; Conover *et al.*, 2009).

In recent years, studies comparing populations across latitudes or altitudes have become more abundant with physiological and morphological traits found to differ along these gradients. For example, plants of lower latitudes were shown to have thicker and more leaves (Etterson, 2004), to flower earlier (Stinchcombe & Weinig, 2004), to be less responsive to red and far-red light (Stenøien *et al.*, 2004) or to have lower nitrogen content (Kudo, 1995). Similar observations have been made along altitudinal gradients with plants from high altitudes having fewer flowers, smaller leaves (Hoffmann *et al.*, 2009) or reduced growth rate (Haider *et al.*, 2012).

Few of these environmental gradient studies looked into the question of phenotypic plasticity at range margins. Maron *et al* (2004) compared the degree of

plasticity between populations of an introduced plant species, *Hypericum perforatum*, at its northern and southern edge of distribution, both in its invaded and home range. They found that both native and introduced plants exhibited substantial plasticity across the cline in both ranges. Plant studies on altitudinal clines often found that plasticity in the field was partially genetically determined (Linhart & M. C. Grant, 1996; Hoffmann *et al.*, 2009; Turpin & Hazard, 2009; Scheepens *et al.*, 2010; Gratani *et al.*, 2012; Morrison & Pickering, 2012; Haider *et al.*, 2012), but the difference in plasticity between core and marginal populations was not directly tested.

In this study, we investigated patterns of trait differences along two latitudinal gradients of *Arabidopsis lyrata* in North America. Many climatic components differ between the North and South including diurnal temperature, wind speed, ground frost frequency or sunshine frequency (New *et al.*, 2002; Parmesan, 2006), but one major environmental variable contrasting across latitudes and habitats is water availability. Therefore, apart from exploring general latitudinal differences among populations, we were interested in the rainfall gradient. Plants from nine populations were exposed to well-watered and dry conditions, so that plastic responses in trait expression could be assessed. The populations covered a cline of 13° latitude, from North Carolina and Missouri, USA, to southern Ontario, Canada. The specific questions we addressed were: Are populations from drier areas better adapted to dry conditions? What are the traits that correlate with latitude and climatic variables? What is the plastic response of populations to drought? And, is phenotypic plasticity higher at range margins?

Materials and Methods

Sampling of Populations

We sampled seeds of 9 populations of *Arabidopsis lyrata* subsp. *lyrata* in 2011 from the USA and southern Canada covering most of the species range (Table 1 and Fig.1). The most southern populations come from North Carolina and Missouri, the northern-most populations from New York State and Lake of the Woods, Ontario. The 9 chosen populations covered two latitudinal gradients, one within each of two ancestral clusters of the species (Fig. 1; Table 1). Molecular work on 19 microsatellites of a parallel study had shown that North American populations of the species fell into two ancestral clusters, an eastern and a western (Griffin and Willi, in prep). The nine selected populations had relatively high expected heterozygosity (range: 0.3-0.6), and low fixation index (F_{is} < 0.1), indicating that the populations harbored high genetic variation and were predominantly outcrossing (Willi & Määttänen, 2010; 2011). A first set of population came from the Midwest: Missouri, Iowa, Wisconsin and southern Ontario (Lake of the Woods). A second set of populations came from the Appalachians and the east coast: North Carolina, Virginia, western Maryland, New Jersey and upstate New York State. For each population, mature fruits of 50 plants were sampled over a surface area of app. 500 m^2.

We assessed the full- or half-sib relationships of field-collected offspring from the same mother plants by microsatellite analysis. For each population, five individuals of two families were raised, their DNA extracted and genotyped at eleven microsatellite loci (for details see Appendix S1 Willi *et al.* (2013)) and scoring was done with the program GeneMapper v. 4.0 (Applied Biosystems). Relatedness of individuals within family was

calculated using the software program ML-Relate (Kalinowski *et al.*, 2006). Maximum likelihood estimates of relatedness were calculated by the downhill-simplex routine as suggested by Wagner *et al.* (2006). Across populations, seeds within families were full-sibs in 70.54% (standard error: 8.71) of the cases and half-sibs in 18.93% (6.85) of the cases.

Climatic data for the sampling sites were extracted from two sources. Worldclim supplied data on average spring temperature and average spring precipitation for the months of April, May and June (Hijmans *et al.*, 2005). The Consortium for Spatial Evolution (www.cgiar-csi.org) supplied data on spring-average actual evapotranspiration, average spring soil-water content and the Priestley-Taylor alpha coefficient, which depicts the general aridity stress on the vegetation (Zomer *et al.*, 2008). We restricted our analysis to the spring period because it corresponds to the growing season of *A. lyrata* (Al-Shehbaz, 2010).

Experimental Design and Raising of Plants

Thirty randomly chosen seed families per population were used for this experiment and grown under two watering treatments: a dry and well-watered/wet treatment (9 populations x 30 families = 270 families). Six individuals per maternal seed family were raised in three blocks, each containing a plant growing under dry conditions and a plant growing under wet conditions (270 maternal seed families x 2 treatments x 3 blocks = 1620 plants). In each block, plants of the two treatments were randomly split into trays (multipot trays of 54 pots with an individual diameter of 4 cm and volume of 63 cm^3; gvz-rossat, Switzerland) and position within tray. At the end, we had 12 trays per block

with 6 per treatment within block (total of 36 trays). Trays were filled with 1:1 mixture of sand and peat, and three small wells were made in each of the 54 pots. Three seeds per pot were sowed, each in a well, to assure that at the end we had at least one plant growing. The wells followed the same spatial arrangement between pots, so that the timing of germination could be attributed to a particular seedling. After sowing, trays were watered with Solbac organic solution (Andermatt Biocontrol SA, Switzerland) in order to prevent the appearance of shoot- and leaf-eating fly larvae. Trays were then put in a cold chamber (4°C, complete darkness and high humidity) for 10 days of stratification. The mix of cold and wet is known to break seed dormancy (Finch-Savage & Leubner-Metzger, 2006). During this process, trays were sprayed with tap water three times a week.

After stratification, trays were placed into three growth chambers (CLF Plant Climatics, Germany) with three levels each (top, middle and bottom). A particular level across the three growth chambers corresponded to one block. Over the whole experiment and once per week, blocks were randomly re-allocated to a level of the three growth chambers and trays within blocks were randomly assigned a position. Germination conditions were set to 8 hours light and 16 hours dark at 18°C with a relative humidity of 40% – 60%. Light intensity was set around 150 µmol m^{-2} s^{-1}. In order to improve germination rate and keep humidity high, trays were covered with a fine-mesh plastic cloth for the first two weeks (Windhager Pflanzenfolie, Austria). Trays were sprayed from above every 1-3 days and watered with Solbac solution from the bottom once a week. After 20 days of germination, settings were changed to imitate longer days with 12 hours light/12 hours dark at 18°C with a light intensity of 200-250 µmol m^{-2} s^{-1}. After

one more week, day length was set to 14 hours at 20°C. We recorded germination every 1-3 days. Twenty days after the first plant germinated, we randomly removed all excess seedlings so that we had one plant per pot.

One month after the beginning of germination, we started applying treatment. Initially, plants of the wet treatment were watered from the bottom twice a week with 1 l of tap water per tray. Plants in the dry treatment were watered with 1 l per tray once a week. By the middle of the week, the soil in the dry treatment revealed extreme drought, although plants did not show reduced growth compared to the well-watered plants. After three weeks, we further reduced watering in the dry treatment and waited for plants to wilt. Once wilted, we gave 6 ml of water per plant, which was repeated every other day for three weeks. To fasten the drying process we also set the conditions to 22°C day temperature. Soil moisture was recorded with a soil moisture probe (Decagon ECH_2O, Pullman, USA) calibrated for soil type and pot size. Volumetric moisture content (VMC) remained under 10% for the dry treatment and above 30% for the wet treatment. All plants were well watered just before trait measurement so that leaf trait measures would not be underestimated (Cornelissen *et al.*, 2003).

Trait Measures

Growth. At the beginning of treatment and throughout, plants were photographed once a week to estimate the growth trajectory. Using a digital camera (Nikon Coolpix P5100, Japan) on a tripod, we recorded size of individual plants by taking pictures of whole trays. We used the ImageJ (Rasband, 2011) software to measure the length of the two longest leaves of each plant. Relative growth rate was calculated by dividing the mean

leaf length at the end of the experiment by the mean leaf length at the third week of treatment.

$\delta^{13}C$. We measured the carbon isotope ratio, which is a measure of integrated water-use efficiency (Farquhar & Richards, 1984). Fresh leaf material (50 mg) was collected from 1085 plants (one complete block and one half each of the two other blocks) and immediately dried in a lyophiliser for 24 hours (Edwards freeze dryer Modulyo, USA). Samples were then ground for 30 seconds with a steel bullet in a milling machine (MM300, Retsch, Germany). Samples were analyzed by isotope mass spectrometry at the University of New Hampshire Stable Isotope Laboratory (Durhman, NH 03824, USA). Final data represent the carbon isotope ratio relative to the standard (R_{PDB}) [$\delta^{13}C$ (‰) = (R_s/R_{PDB}-1) x 1000] (Farquhar & Ehleringer, 1989). We corrected the data for the ambient ^{12}C:^{13}C ratio by subtracting the average $\delta^{13}C$ obtained from 8 corn plants, which were raised over the treatment period with the experimental plants, distributed over the three blocks. Corn utilizes C4 versus C3 metabolism, and it does not discriminate between the two carbon isotopes. Therefore, the carbon isotope ratio in its tissue is a useful reference for the ambient carbon isotope ratio.

Stomata density and stomata length. We measured stomata density and length using one fully extended and hardened leaf per plant. Using clear nail varnish, we polished a small portion of the abaxial side of the leaf and let it dry for about five minutes. Once dried, we affixed a small piece of clear tape over the polished portion. The tape was removed, leaving the dried nail polish with the stomata impressions stuck on it. The tape was then affixed to a clean microscope slide (Thermo Scientific, USA). Stomata impressions were photographed at x100 magnification using a camera attached to a

microscope (Leica Microsystems GmbH Wetzlar, Germany) and analyzed using ImageJ (Rasband, 2011). Stomata density was counted over a surface of 206'822 μm^2 and then divided by dry matter (dry mass of leaf portion/surface area of leaf portion) in order to obtain stomata density per mg dry weight. We calculated stomata length as the average distance in micrometers between the guard-cell junctions of 10 stomata (Maherali *et al.*, 2002). Based on the average size of the stomata and the surrounding epidermis cells, we made sure that measurements were taken on stomata of about the same age.

Leaf dissection index. Two fully extended and hardened leaves were collected from each plant (1620 plants x 2 leaves = 3240 measures). As described before, leaves were of the same age and had grown during the treatment period. Sampled leaves were scanned (HP Scanjet G2710) and analyzed using ImageJ (Rasband, 2011) to obtain measures of leaf perimeter and leaf area. Leaf dissection index (DI) was calculated using the Fourier transformation method described by Kincaid and Schneider (1983): $DI = perimeter/(2\sqrt{(area \times \Pi)})$. The index value is without unit but comparable to a circle with a DI of 1.

Leaf-dry-matter content. Prior to scanning, a disc was punched out from the distal end of the leaf blade, along the central vein. If a leaf was of average size, the diameter of the disc was 0.6 cm. For small leaves, we used a hole-punch of 0.3 cm diameter. The discs were immediately weighed with a precision balance (Mettler Toledo XA204DR). Then each disc was placed into a white unbleached paper bag (Flachbeutel SKB559, Germany) and dried in an oven at 60°C for 48 hours. Dry mass of each disc was then taken on a microscale (Mettler Toledo XP6, USA). Leaf-dry-matter content (LDMC) was calculated for two leaves per plant as the oven dry mass of the disc (mg) divided by its

water-saturated fresh mass (g), expressed in mg g^{-1} (Cornelissen *et al.*, 2003). The averaged LDMC of the two leaves was used for the experiment.

Trichome density. We calculated trichome density by counting the total number of trichomes on the two discs of a plant and divided the number by disc area. Counting was done just before the discs were dried, directly after measuring wet weight.

Flowering time. Once plants started flowering, we recorded the day of first flower opening every 2-3 days throughout the flowering period. Prior to calculating flowering time, germination and flowering dates were adjusted to the mid-point of checking in order to be as close as possible to the true date. Flowering time was calculated as the number of days between germination and the appearance of the first flower. Flowering time for plants that did not flower was calculated as the number of days between germination and the end of the experiment plus: 2 days for plants with big flower buds, 7 days for bolted plants with long stem, 20 days for bolted plants with short stem and 40 days for unbolted plants.

Statistical Analysis

All analyses were performed using the R software (R Development Core Team, 2012). Fist, we correlated environmental variables against each other to see whether there was some grouping. Second, we explored the effects of treatment, genetic cluster and latitude on phenotypic data. This analysis was completed with a linear mixed model (lme4 package: Bates *et al.*, 2012) with restricted maximum likelihood (REML) and the following hierarchical structure of random effects: plant nested within family and population at the first level, family nested within population at the second level, and

population at the third level (Singer, 1998; Fox, 2002). Treatment was a predictor variable on the level of the plant, genetic cluster was a predictor variable on the level of the population, and latitude was a predictor covariate on the level of the population. Covariates were centered to a mean of 0. Prior to the analysis, all traits were corrected for tray effect within treatment. Furthermore, to approach normality of the residuals, the following response variables were log-transformed: relative growth, flowering time and trichome density. Third, we explored patterns of trait plasticity calculated as family mean in the dry treatment minus family mean in the wet treatment. Therefore, plasticity was calculated for each family, resulting in 270 measures for each trait. We performed a mixed model analysis (lme4 package: Bates *et al.*, 2012) with restricted maximum likelihood (REML) to evaluate the effect of latitude, the square-term of latitude (latitude^2 = latitude*latitude), genetic cluster and cline position on our plasticity measure. In this model, latitude, latitude2, genetic cluster and cline position were covariates on the level of population with population as random effect. Cline position had two levels: central and edge (central: Missouri, North Carolina, Virginia; edge: Wisconsin, Lake of the Woods, New York).

Results

Correlation between latitude and climatic variables. Latitude was strongly negatively correlated with average spring climatic variables (Table 2). High latitude sites were associated with lower temperature, lower precipitation and lower actual evapotranspiration. But, latitude was not significantly correlated either with spring soil-water content or general aridity. However the latter two were strongly positively

correlated. Based on these patterns, we only considered latitude as an environmental predictor variable in further analysis.

The effect of treatment and latitude. Mixed-model analyses showed that all traits except leaf dissection and stomata density were strongly affected by treatment, implying that treatment induced pronounced plastic trait differences (Table 3). Under drought, plants flowered earlier, grew slower, had denser leaves, smaller stomata, more trichomes and higher $\delta^{13}C$, indicating a greater water-use efficiency (Fig. 2). Flowering time was significantly associated with latitude, and $\delta^{13}C$ showed a trend. Plants from the North flowered about seven weeks earlier and were, to some extent, more water-use efficient (difference of ± 2 between max and min values) than those from the South (Fig. 3). As expected, a parallel trend appeared with spring precipitation (averages per month): plants flowered earlier and seemed more water use efficient with lower precipitations (Fig.4; t = 1.96 and -1.82 respectively, $Pval < 0.1$). The interaction between treatment and latitude revealed a trend for leaf dissection index and was significant for relative growth. While leaf dissection tended to increase with latitude in the dry treatment more strongly than in the wet treatment, growth increased with latitude more strongly in the wet than in the dry treatment. No trait differed significantly between the eastern and western genetic clusters.

Plastic responses at center versus margins of distribution. Only leaf dissection index showed a significant relationship between plasticity, latitude and latitude^2, the square-term of latitude (Table 4). Therefore, while plasticity in leaf dissection index has a linear relationship with latitude, less plasticity was present at distribution edges (Fig.5). Position along the cline was significantly associated with plasticity in leaf dissection index, leaf-dry-matter content and revealed a trend for stomata length. Therefore,

plasticity in these traits seemed to depend on whether populations were in central or marginal locations. Plasticity in water-use-efficiency had a significant relationship with genetic cluster. A similar trend appeared for stomata density and stomata length. In addition, cline position and genetic cluster interacted in their effect on plasticity for relative growth; populations of the western cluster had reduced plasticity at range margins in growth compared to central populations. Overall, while populations at the edges of the distribution did not show heightened levels of plasticity, populations from the western and eastern cluster appeared to differ in their plastic response to drought (Fig. 5). In particular, western populations showed a larger change in flowering time, leaf dissection index, growth and $\delta^{13}C$ than eastern populations: in western populations, plasticity in flowering time was greater at distribution edges but plasticity in leaf dissection, relative growth and $\delta^{13}C$ was smaller than in central locations.

Discussion

Space-for-time substitution studies (Pickett, 1989) performed in a common garden environment have the potential to reveal the traits of adaptation along climatic clines. When conditions are manipulated to imitate aspects of climate change, plastic responses to the changing conditions can be estimated. Many studies have focused on temperature adaptation along latitudinal or altitudinal gradients (Kozlowski & Pallardy, 2002; Davey *et al.*, 2009; Manel *et al.*, 2010; Keller & Seehausen, 2011), but research linking adaptive and plastic responses to water availability across latitudes are rare.

In this study we found signs of potential adaptation to water availability along latitudinal gradients. First, spring temperature and precipitation were negatively

correlated with latitude within the geographic distribution of *Arabidopsis lyrata* subsp. *lyrata*, indicating dryer conditions as we approach northern boundaries. Flowering time was significantly associated with latitude independent of treatment, while water-use efficiency showed a trend. Plants from the North flowered earlier, and to some extent, they were more water-use efficient. Plastic responses to drought also included earlier flowering and more water-use efficiency. These observations are in line with previous findings on *Arabidopsis thaliana*, *Avena barbata* or *Brassica rapa* (Mckay *et al.*, 2003; Sherrard *et al.*, 2009; Franks, 2011). Therefore, the environmental response to drier conditions and the genetic difference from wetter to drier sites co-varied, and adaptations were fixed, providing evidence of co-gradient variation (Conover & Schultz, 1995).

The experimental treatment revealed further plastic responses to drought. Under dry conditions, plants grew more slowly, had higher leaf-dry-matter content, shorter stomata and higher trichome density. Slower growth can be interpreted as a stress response and thus cannot be considered adaptive (Harb *et al.*, 2010). However, all other traits seemed to change in the direction expected for an adaptive response. Greater leaf dry-matter content means that more water is retained per dry mass. Shorter stomata can close faster, leading to lower water loss under drought (Hetherington & Woodward, 2003). Also, greater trichome density allows the build-up of an intermittent zone of elevated moisture between the inner of the leaf and the air, further reducing water-loss (Picotte *et al.*, 2009). Shorter flowering time has also been shown to be adaptively favored under drought, and it has been considered a strategy of drought escape as opposed to drought tolerance (Franks & Weis, 2008). Overall, populations of *A. lyrata*

show high phenotypic plasticity in a number of traits in response to drought, always in the direction expected to be adaptively favored.

The association of early flowering and heightened water-use efficiency contrasts with findings of drought adaptation in *A. thaliana*. Studies on *A. thaliana* found that plants from northern latitudes generally flowered earlier, but were less water-use efficient (Stinchcombe & Weinig, 2004; Anderson *et al.*, 2011). In that species, flowering time and water-use efficiency were also shown to be genetically negatively correlated (Caicedo *et al.*, 2004). The discrepancy between those and our findings may be explained by the fact that both shorter vegetation period and dryer conditions in the North selected for early flowering, and drier conditions additionally selected for higher water-use efficiency. Also no genetic correlation between flowering time and water-use efficiency was found for *A. lyrata* subsp. *lyrata* growing across heterogeneous sand dunes habitats (Paccard *et al.*, submitted).

Empirical evidence of co-gradient variation remains rare, particularly in plant species (Conover *et al.*, 2009). Our results provide strong evidence of co-gradient variation for flowering time and water-use efficiency in *A. lyrata* as genetic and the environmental influences on the phenotype seemed to act in the same direction (Conover & Schultz, 1995; Conover *et al.*, 2009). Co-gradient variation has been defined as a form of adaptive phenotypic plasticity evolving through co-gradient selection. In this case, one evolutionary implication of such variation is the promotion of range expansion: establishing individuals to new habitats would express traits' values to be partly advantaged and may posses genetic variation for adaptation to this new location (Ghalambor *et al.*, 2007; Conover *et al.*, 2009). Hence, while we are lacking evidence

that responses in flowering time and water-use efficiency enhance fitness at range limits, we could imagine that the observed co-gradient variation could facilitate expansion beyond the distribution margins.

Adaptation by phenotypic plasticity may be an alternative to fixed adaptive differences for populations living in marginal habitats where multiple evolutionary constraints are present (Moczek *et al.*, 2011). Swamping of adaptive alleles by gene flow from the center to range margins may create maladaptation, leading to strong directional selection for the evolution of plasticity (Chevin & Lande, 2011). However, our results indicate that plasticity is not necessarily greater at range margins but rather varies across the clines depending on the traits. The model of Chevin and Lande (2011), like many other range margin models (reviewed by: Kawecki, 2008; Sexton *et al.*, 2009) assumes substantial gene flow from the core to the marginal populations. In herbaceous species with little long-distance seed dispersal, this assumption is unlikely over large geographic ranges. These *A. lyrata* populations are highly genetically differentiated, pointing to restricted rather than ongoing gene flow between them (Griffin and Willi, in prep). Thus, while Chevin & Lande's model (2011) offers insight into the evolution of plasticity at range margins, it might not be sufficient to explain patterns of plasticity across large ecological clines. Instead, we suggest that the varying patterns observed may be the result of (1) restricted gene flow between populations, (2) greater genetic variation in central locations (Gillespie & Turelli, 1989; Goldstein & Holsinger, 1992; Griffin and Willi, in prep) or, (3) a non-linear relationship between latitude and water availability.

In this study, plants were raised from seeds directly harvested on the studied sites. Therefore, the observed phenotypic differences across populations bred in a common

environment could be the result of non-genetic maternal effects. However, there is little evidence that adaptive maternal effects drive patterns of phenotypic differentiation along large geographic clines. In fact, when environmental variation is large enough so that gene flow would not hinder local adaptation, adaptive maternal effects are not expected (Galloway, 2005; Montague *et al.*, 2008). In our study gene flow between populations is known to be very low (Willi & Määttänen 2010), so the large-scale latitudinal differentiation observed in this study is unlikely to be the consequence of maternal effects. This is particularly true for traits like flowering time where the genetic basis of cline variation in *Arabidopsis thaliana* has been well identified (Stinchcombe & Weinig, 2004).

Studying evolution over large latitudinal gradients can help us understand species adaptation to rapid environmental change. In this study, we found two traits potentially related to climate adaptation: flowering time and water-use efficiency. Plastic response to drought did not appear to be greater at distribution's edges. Instead, plasticity seemed to vary by trait across the entire range of *Arabidopsis lyrata*. Among other explanations, this may be the result of restricted gene flow between populations, favoring fixed trait differences in adaptation to local climate. Exploring trait variation and trait integration will allow us to investigate whether limits to distribution are caused by genetic variation or genetic correlations.

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Table 1. List of *Arabidopsis lyrata* populations studied along a western and an eastern latitudinal cline, with information on location, genetic cluster, cline position, latitude and longitude.

Population	Location	Genetic cluster	Cline position	Latitude, N	Longitude, W
A	Fort Leonard Wood	West	Edge	37° 43'	92° 03'
B	Malanaphy Springs State Preserve	West	Central	43° 21'	91° 50'
C	Namekagon Barrens Wildlife Area	West	Edge	46° 06'	92° 04'
D	Lake of the Woods	West	Edge	49° 39'	94° 55'
E	Mayodan South	East	Edge	36° 24'	79° 57'
F	Fort Hill Preserve	East	central	39° 29'	78° 55'
G	Clark Reservation	East	Edge	42° 59'	76° 05'
H	Providence Forge	East	Edge	37° 25'	77° 01'
I	Sandy Hook	East	central	40° 26'	73° 59'

Table 2. Pearson correlation matrix of latitude and spring climatic variables of sampling sites (averages April to June): temperature, precipitation, actual evapotranspiration, soil-water content and overall aridity, the latter for the whole year. Correlation coefficients (*r*) are reported. * P < 0.05, ** P < 0.01. Coefficients with *P* < 0.05 are in bold.

	Latitude	Temperature	Precipitation	Evapotranspiration	Soil-water content	Aridity
	r	*r*	*r*	*r*	*r*	*r*
Temperature	**-0.97****					
Precipitation	**-0.84***	**0.84****				
Evapotranspiration	**-0.98****	**0.95****	**0.87****			
Soil-water content	-0.33	0.22	0.33	0.33		
Aridity	-0.26	0.20	0.26	0.23	**0.93****	

Table 3. Results of mixed model analysis testing the effect of treatment (T; dry versus wet), source latitude (L), their interaction and genetic cluster (west versus east) on all traits. *t*-values are reported. Significant values are indicated: (*) P < 0.1, * P < 0.05, ** P < 0.01. *t*-values with *P* < 0.1 are in bold. Random effects are not shown.

	Treatment	Latitude	T*L	Cluster
Flowering time	**2.29***	**-2.02***	-0.29	-0.64
Dissection index	-0.11	1.18	**-2.01(*)**	-1.24
Relative growth	**5.95****	0.44	**2.87***	1.22
Leaf-dry-matter content	**-6.67****	-0.32	0.84	-0.41
Stomata density	-1.50	-1.00	-1.01	1.89
Stomata length	**9.49****	0.18	-0.64	-0.54
Trichome density	**-15.04****	0.26	-1.06	-1.28
Water-use efficiency ($\delta^{13}C$)	**-46.31****	**1.84(*)**	-1.25	-1.04

Table 4. Results of mixed-model analysis testing the effect of latitude, latitude^2 (the square-term of latitude), genetic cluster, cline position (edge versus central) and their interaction on the plastic response of each trait (difference in family mean trait value between dry and wet treatment). *t*-values are represented. Significant values are indicated: (*) P < 0.1, * P < 0.05, ** P < 0.01. *t*-values with *P* < 0.1 are in bold.

	Latitude	Latitude^2	Cline position	Cluster	Cline position*Cluster
Flowering time	1.26	-1.19	-1.27	-1.22	0.67
Dissection index	**3.76****	**-3.66****	**-2.54***	-0.46	-0.68
Relative growth	-0.76	0.73	0.13	-0.42	**2.95****
Leaf-dry-matter content	0.66	-0.64	**-3.2****	-1.43	1.47
Stomata density	0.02	0.09	0.92	**-2.17(*)**	-0.55
Stomatal length	-0.32	0.27	**-1.92(*)**	**2.06(*)**	1.82
Trichome density	1.71	-1.62	0.12	-1.5	-0.33
Water-use efficiency ($\delta^{13}C$)	0.92	-0.8	-0.05	**-2.7***	-1.33

Figure legend

Figure 1. Locations of the *Arabidopsis lyrata* populations from both western (black triangles) and eastern (black circles) latitudinal gradients. The two gradients depict two different ancestral genetic clusters (Griffin and Willi, in prep).

Figure 2. Mean performance of *Arabidopsis lyrata* populations under well-watered (black dots) and dry conditions (grey dots). Population means (calculated from family means) and regression lines are represented. Overall treatment means are represented by stars (black and grey for wet and dry treatments respectively) connected by dashed lines. All variables shown are untransformed, though flowering time, relative growth and trichome density were log-transformed for statistical analysis.

Figure 3. Mean performance of *Arabidopsis lyrata* populations under wet (black dots) and dry conditions (grey dots), over latitude. Population means and standard deviations are based on family means. Regression lines with a slope significantly different from zero are shown for the two treatments, wet (continuous line) and dry (dashed line). All variables shown are untransformed, but flowering time, relative growth and trichome density were log-transformed for statistical analysis.

Figure 4. Flowering time and water-use efficiency (δ13C) responses of *Arabidopsis lyrata* population under wet (black dots) and dry conditions (grey dots), depending on spring precipitations. Population means and standard deviations are based on family

means. Regression lines with a slope significantly different from zero are shown for the two treatments, wet (continuous line) and dry (dashed line)

Figure 5. Means of absolute values of plasticity from populations of northern, southern and central locations. Both eastern (black dots connected by continuous line) and western (black triangles connected by dashed line) genetic clusters are represented. Population means average per cline location and standard errors are represented.

Figure 1

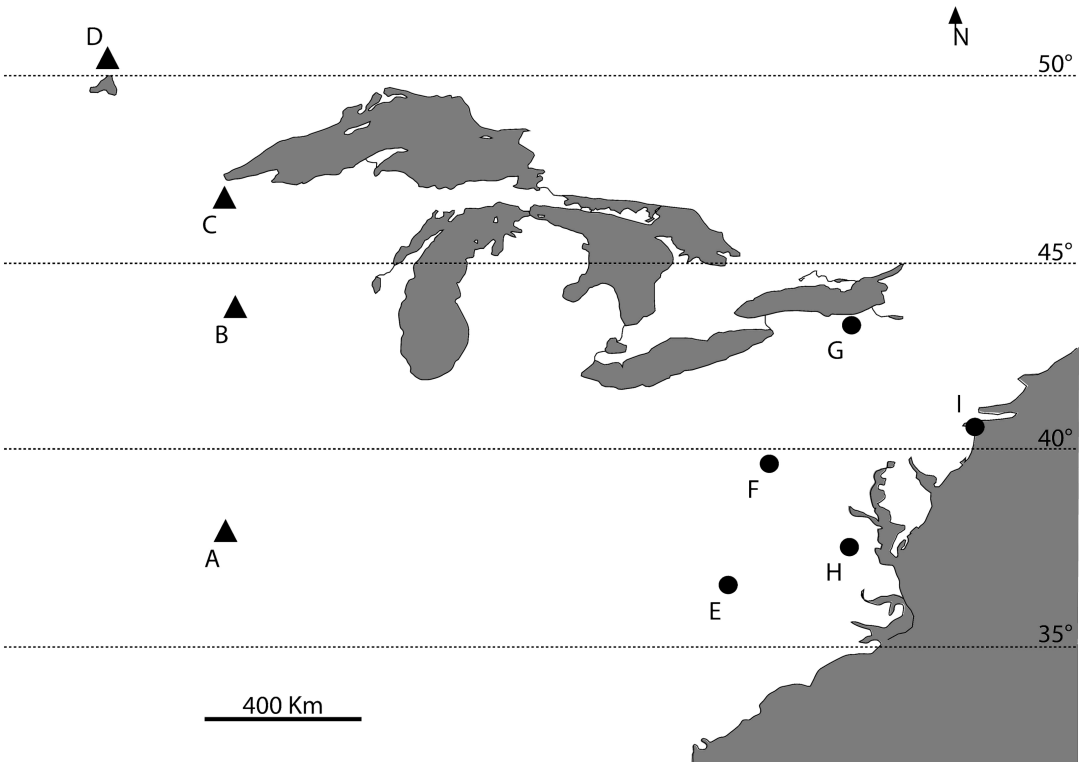


Figure 2

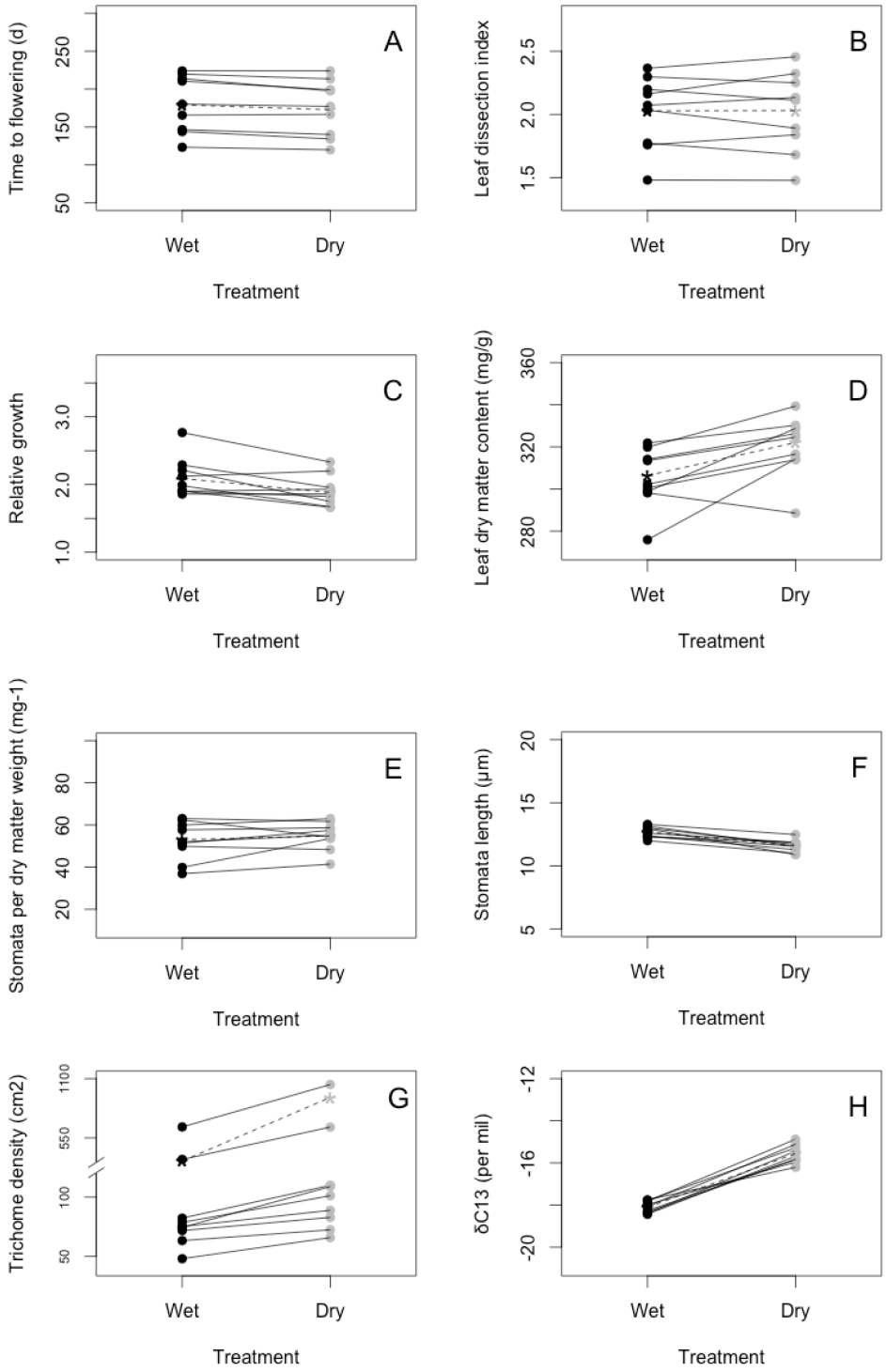

A
Time to flowering (d)
50
150
250
Wet
Dry
Treatment
B
Leaf dissection index
1.5
2.0
2.5
Wet
Dry
Treatment
C
Relative growth
1.0
2.0
3.0
Wet
Dry
Treatment
D
Leaf dry matter content (mg/g)
280
320
360
Wet
Dry
Treatment
E
Stomata per dry matter weight (mg-1)
20
40
60
80
Wet
Dry
Treatment
F
Stomata length (µm)
5
10
15
20
Wet
Dry
Treatment
G
Trichome density (cm2)
50
100
550
1100
Wet
Dry
Treatment
H
δC13 (per mil)
-20
-16
-12
Wet
Dry
Treatment

Figure 3

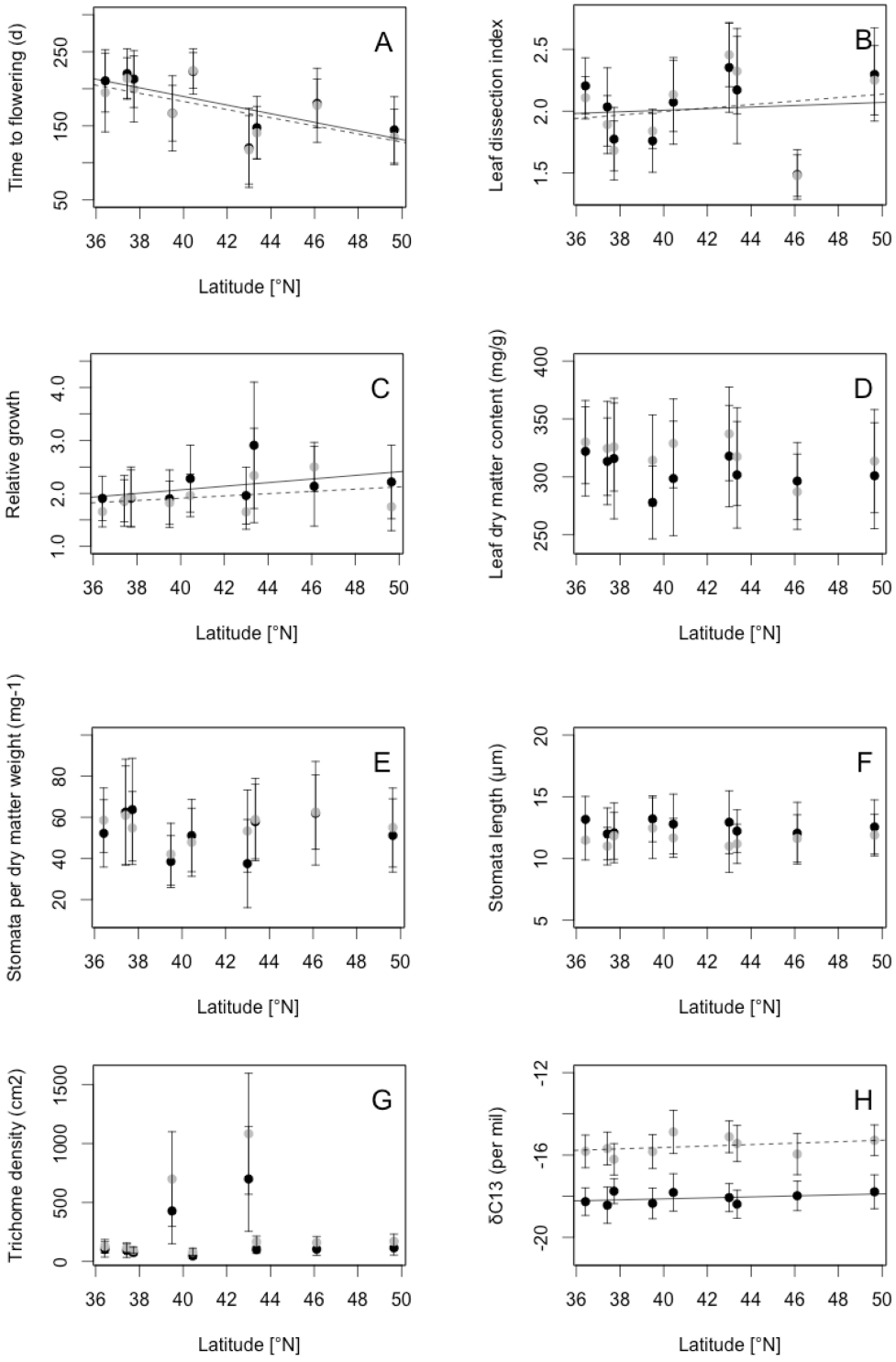

A
Time to flowering (d)
50
150
250
36 38 40 42 44 46 48 50
Latitude [°N]
B
Leaf dissection index
1.5
2.0
2.5
36 38 40 42 44 46 48 50
Latitude [°N]
C
Relative growth
1.0
2.0
3.0
4.0
36 38 40 42 44 46 48 50
Latitude [°N]
D
Leaf dry matter content (mg/g)
250
300
350
400
36 38 40 42 44 46 48 50
Latitude [°N]
E
Stomata per dry matter weight (mg-1)
20
40
60
80
36 38 40 42 44 46 48 50
Latitude [°N]
F
Stomata length (µm)
5
10
15
20
36 38 40 42 44 46 48 50
Latitude [°N]
G
Trichome density (cm2)
0
500
1000
1500
36 38 40 42 44 46 48 50
Latitude [°N]
H
δC13 (per mil)
-12
-16
-20
36 38 40 42 44 46 48 50
Latitude [°N]

Figure 4

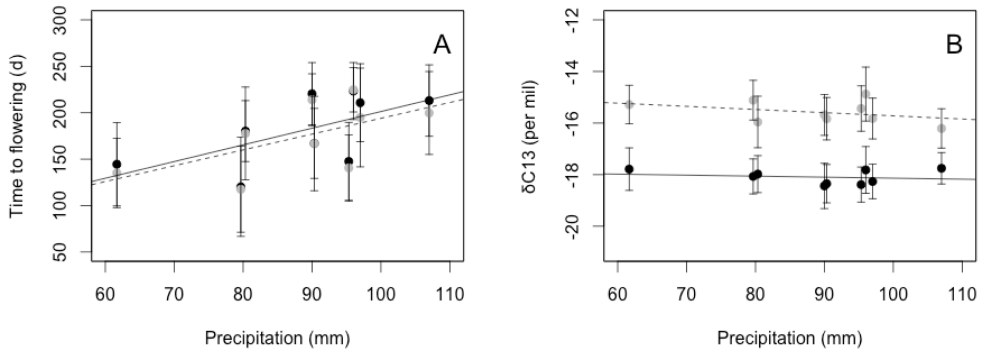
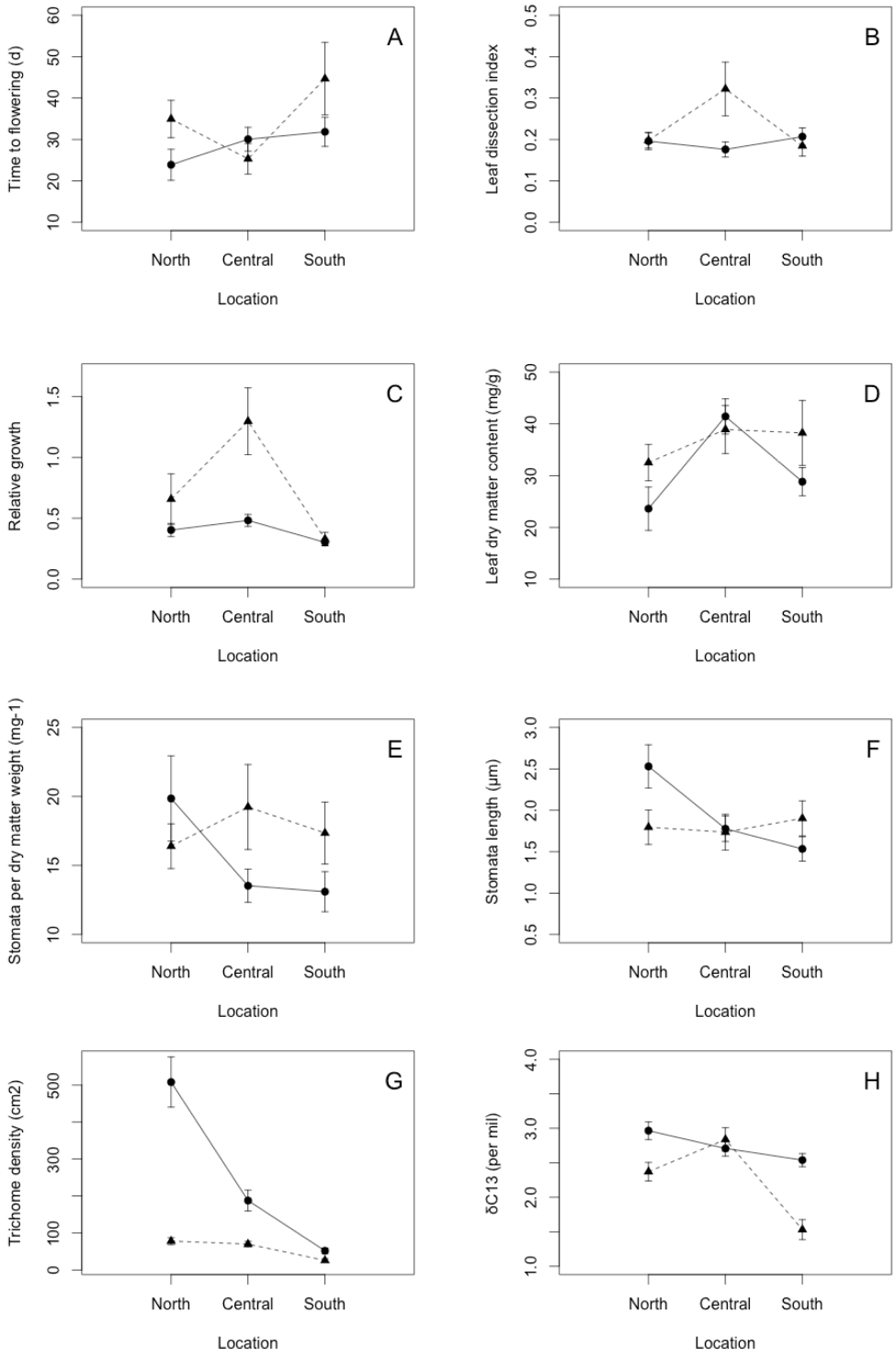

A
Time to flowering (d)
50
100
150
200
250
300
60
70
80
90
100
110
Precipitation (mm)
B
δC13 (per mil)
-20
-18
-16
-14
-12
60
70
80
90
100
110
Precipitation (mm)

Figure 5


A
Time to flowering (d)
10 20 30 40 50 60
North Central South
Location
B
Leaf dissection index
0.0 0.1 0.2 0.3 0.4 0.5
North Central South
Location
C
Relative growth
0.0 0.5 1.0 1.5
North Central South
Location
D
Leaf dry matter content (mg/g)
10 20 30 40 50
North Central South
Location
E
Stomata per dry matter weight (mg-1)
10 15 20 25
North Central South
Location
F
Stomata length (µm)
0.5 1.0 1.5 2.0 2.5 3.0
North Central South
Location
G
Trichome density (cm2)
0 100 300 500
North Central South
Location
H
δC13 (per mil)
1.0 2.0 3.0 4.0
North Central South
Location

Chapter 3. A comparison of trait integration under drought stress along latitudinal clines in *Arabidopsis lyrata*

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Abstract

One of the most exciting open questions in evolutionary biology is why species have spatially restricted distributions. There are two sorts of hypotheses: biotic players restrict the realized niche or that species lack mutations allowing for adaptation to more environmental extremes. In this study, we asked whether plants growing at the northern or southern boundaries of the distribution are more constrained in their adaptation to drought. We raised plants from replicate seed families of nine *Arabidopsis lyrata* populations covering two latitudinal gradients of 13°. Plants were subjected to dry and control conditions. Several traits, related to the plants' water balance were measured. Broad-sense genetic variance-covariance matrices were built for each population and treatment and then compared. Populations of both the southern and northern edges of the distribution had lower levels of genetic variation compared to central populations. Pairwise comparisons between central and marginal populations revealed that two northern populations had a reduced effective number of dimensions reflecting stronger patterns of constraints. In addition, the angle between the multivariate selection vector and the vector of predicted selection response was significantly greater for populations from the northern boundaries than those from central locations under control conditions. These observations documents that the genetic architecture of northern populations restricts adaptive evolution more strongly than that in more southern populations. Therefore, the evolutionary potential of *Arabidopsis lyrata* showed restrictions at the leading edge of distribution compared to the center and the trailing edge.

Keywords: Range margins, latitudinal gradients, G matrix, *Arabidopsis lyrata*.

Introduction

An open question in evolutionary biology is why species have restricted distributions, and why they cannot adapt to overcome them. From a purely ecological point of view, every species occurs over a spatial or temporal range corresponding to its specific ecological niche. Hutchinson (1957; 1961) defined the concept of the ecological niche as the set of conditions and resources required and/or tolerated by a species' population for its survival. Following this concept, logic would dictate that populations should only occur where niche's requirements are met. However, observation of species distribution tells us that this is not necessarily true (Pulliam, 2000; Kawecki, 2008). Populations may be absent because colonization has not occurred, or populations may be maintained outside the niche by immigration. Such marginal habitats occur at the boundary of the ecological niche and should be identified as spars transition areas between the core habitats (suitable) and less suitable ones (Kawecki, 2008). Adding an evolutionary perspective to the niche, it can be expected that a population adapts to marginal conditions, engaging in the evolution of its niche. However, there must be clear limits to niche evolution because species have generally clear edges within and at the outer boundary of the species range. Here we investigated potential traits and their genetic integration that could limit the distribution of *Arabidopsis lyrata*.

Theory suggests that several factors are important in explaining limitation to species distribution at range margins. While different modes of selection may be present between the core and edges habitats (Hoffmann & Blows, 1994; Bridle & Vines, 2006; Kawecki, 2008), stabilizing selection may be more common in central locations and strong directional selection may predominate at the edge of the distribution (Hutchinson,

1957; 1961; Sexton *et al.*, 2009). Additive genetic variance in traits under selection (V_A) will be critical at range margins for an adaptive response to occur (Fisher, 1930; Pulliam, 2000; Kawecki, 2008). However, erosion of genetic variation due to strong selection or genetic drift in marginal populations are expected to slow down adaptive evolution (Fisher, 1930; Wright, 1931; Kawecki, 2008). A further limiting factor may be gene flow, though its effect is controversially discussed. Some theoretical models suggest that adaptation to range limits is more likely to happen when genetic variation is increased by migration (Holt & Gaines, 1992; Hoffmann & Blows, 1994; Holt, 2003; Bridle & Vines, 2006). Other models predict the contrary: only in the absence of strong gene flow from the core to peripheral populations, can genetic variance increase populations' survival (Lynch & Lande, 1993; Bürger & Lynch, 1995; Kirkpatrick & Barton, 1997). Predictions seem to depend on the extend to which specific alleles will have on fitness (Kawecki, 2008) and the steepness of the environmental gradient (reviewed by Sexton and colleagues, 2009). In summary, intense directional selection, low genetic variation and either too little or too much gene flow are predicted to limit adaptive evolution at range margins.

Most of this theoretical work focused on individual traits with some simple genetic architecture. However, adaptation to range margins is likely to involve many traits that sometimes show complex interactions. In this context, understanding the process of adaptation to marginal habitats should be tackled in a multivariate perspective, e.g., by use of the variance-covariance matrix (G). The G-matrix is a mathematical representation of variances and covariances (diagonal and off-diagonal cells, respectively) among quantitative traits and can be constructed for inferences of genetic

constraints (Lande, 1979). In this regard, adaptation to marginal habitats could be obstructed because of strong interdependence between traits due to pleiotropy and epistasis (Kawecki, 2008). Comparisons of G matrices among natural populations or treatments is an area of active research (Shaw, 1991; Phillips & Arnold, 1999; Roff *et al.*, 1999; Mcguigan, 2005; Simonsen & Stinchcombe, 2010), and constraints can be evaluated by comparing the orientations of the vector of multivariate selection with the main axes of G (Blows *et al.*, 2004; Blows & Hoffmann, 2005). So far, almost no empirical work has investigated the evolution of G over large ecological gradients and its implication at range margins. In particular, the lack of such studies at northern expansion edges make it difficult to perceive whether the observed patterns are characteristic of marginal populations or if they are the result of post glacial range expansion (Eckert *et al.*, 2008; Sexton *et al.*, 2009).

There are some examples of climate change research over latitudes that have chosen a multivariate approach. For example, Etterson (2004a; b) studied the evolutionary potential to climate change in the annual prairie legume *Chamaecrista fasciculata*. Three different populations were reciprocally transplanted to three environments across a latitudinal gradient in the Great Plains of North America, and the G-matrices were constructed for each population at each site. The authors found that the northern population might be challenged in its adaptation to climate change due to low heritability, demographic instability and cross-environment genetic correlations perpendicular to selection. In this case, predicted rates of evolutionary responses appeared to be much slower than the predicted rate of climate change. More recently and in a similar context, Colautti and Barrett (2011) investigated patterns of divergence in the

G-matrices of the invasive plant *Lythrum salicaria* across a latitudinal gradient in North America. They investigated genetic constraints on life history traits in 20 populations grown under similar conditions in a greenhouse. They found that while genetic variances were significant for all measured traits, intercorrelations among fitness traits revealed trade-offs, constraining population divergence. This observation was supported by high similarity of the genetic variance-covariance matrix of each population and the matrix of covariance among population means. They concluded that this species had been strongly constrained in its invasion by strong correlations in life history traits.

In our study, we investigated patterns of genetic architecture along two latitudinal gradients in the North American species *Arabidopsis lyrata*. Populations were sampled from North Carolina and Missouri, USA, to southern Ontario, Canada, thus covering a cline of 13° latitude. The latitudinal gradient depicts – besides a strong mean temperature gradient – a gradient in water-availability (Paccard *et al*, in prep). This gradient seems non linear; in the South, water-availability is limited most via high temperatures, and in the North, water-availability is limited most via lower precipitation (Paccard *et al*, in prep). Therefore, we exposed plants from nine populations to two watering treatments: well-water and dry conditions. Three sets of questions were addressed: A. Are populations from the edge of distribution more constrained in adaptive evolution? Have they lower broad-sense genetic variances? Do their G-matrices indicate potential limits to adaptation? B. Do G-matrices of drought conditions show a stronger signature of limits to drought adaptation?

Materials and Methods

We sampled seeds of 9 populations of *Arabidopsis lyrata* subsp. *lyrata* in 2011 from the USA and southern Canada covering most of the species range (Table 1 and Fig.1). The most southern populations come from North Carolina and Missouri, the northern-most populations from New York State and Lake of the Woods, Ontario. The 9 chosen populations covered two latitudinal gradients, one within each of two ancestral clusters of the species (Fig. 1; Table 1). Molecular work on 19 microsatellites of a parallel study had shown that North American populations of the species fell into two ancestral clusters, an eastern and a western (Griffin and Willi, in prep). The nine selected populations had relatively high expected heterozygosity (range: 0.3-0.6), and low fixation index (F_{is} < 0.1), indicating that the populations harbored high genetic variation and were predominantly outcrossing (Willi & Määttänen, 2010; 2011). A first set of population came from the Midwest: Missouri, Iowa, Wisconsin and southern Ontario (Lake of the Woods). A second set of populations came from the Appalachians and the east coast: North Carolina, Virginia, western Maryland, New Jersey and upstate New York State. For each population, mature fruits of 50 plants were sampled over a surface area of app. 500 m^2.

We assessed the full- or half-sib relationships of field-collected offspring from the same mother plants by microsatellite analysis. For each population, five individuals of two families were raised, their DNA extracted and genotyped at eleven microsatellite loci (for details see Appendix S1 Willi *et al.* (2013)) and scoring was done with the program GeneMapper v. 4.0 (Applied Biosystems). Relatedness of individuals within family was calculated using the software program ML-Relate (Kalinowski *et al.*, 2006). Maximum

likelihood estimates of relatedness were calculated by the downhill-simplex routine as suggested by Wagner *et al.* (2006). Across populations, seeds within families were full-sibs in 70.54% (standard error: 8.71) of the cases and half-sibs in 18.93% (6.85) of the cases.

Experimental Design and Raising of Plants

Thirty randomly chosen seed families per population were used for this experiment and grown under two watering treatments: a dry and well-watered/wet treatment (9 populations x 30 families = 270 families). Six individuals per maternal seed family were raised in three blocks, each containing a plant growing under dry conditions and a plant growing under wet conditions (270 maternal seed families x 2 treatments x 3 blocks = 1620 plants). In each block, plants of the two treatments were randomly split into trays (multipot trays of 54 pots with an individual diameter of 4 cm and volume of 63 cm^3; gvz-rossat, Switzerland) and position within tray. At the end, we had 12 trays per block with 6 per treatment within block (total of 36 trays). Trays were filled with 1:1 mixture of sand and peat, and three small wells were made in each of the 54 pots. Three seeds per pot were sowed, each in a well, to assure that at the end we had at least one plant growing. The wells followed the same spatial arrangement between pots, so that the timing of germination could be attributed to a particular seedling. After sowing, trays were watered with Solbac organic solution (Andermatt Biocontrol SA, Switzerland) in order to prevent the appearance of shoot- and leaf-eating fly larvae. Trays were then put in a cold chamber (4°C, complete darkness and high humidity) for 10 days of stratification. The mix of cold and wet is known to break seed dormancy (Finch-Savage

& Leubner-Metzger, 2006). During this process, trays were sprayed with tap water three times a week.

After stratification, trays were placed into three growth chambers (CLF Plant Climatics, Germany) with three levels each (top, middle and bottom). A particular level across the three growth chambers corresponded to one block. Over the whole experiment and once per week, blocks were randomly re-allocated to a level of the three growth chambers and trays within blocks were randomly assigned a position. Germination conditions were set to 8 hours light and 16 hours dark at 18°C with a relative humidity of 40% – 60%. Light intensity was set around 150 µmol m^{-2} s^{-1}. In order to improve germination rate and keep humidity high, trays were covered with a fine-mesh plastic cloth for the first two weeks (Windhager Pflanzenfolie, Austria). Trays were sprayed from above every 1-3 days and watered with Solbac solution from the bottom once a week. After 20 days of germination, settings were changed to imitate longer days with 12 hours light/12 hours dark at 18°C with a light intensity of 200-250 µmol m^{-2} s^{-1}. After one more week, day length was set to 14 hours at 20°C. We recorded germination every 1-3 days. Twenty days after the first plant germinated, we randomly removed all excess seedlings so that we had one plant per pot.

One month after the beginning of germination, we started applying treatment. Initially, plants of the wet treatment were watered from the bottom twice a week with 1 l of tap water per tray. Plants in the dry treatment were watered with 1 l per tray once a week. By the middle of the week, the soil in the dry treatment revealed extreme drought, although plants did not show reduced growth compared to the well-watered plants. After three weeks, we further reduced watering in the dry treatment and waited for plants to

wilt. Once wilted, we gave 6 ml of water per plant, which was repeated every other day for three weeks. To fasten the drying process we also set the conditions to 22°C day temperature. Soil moisture was recorded with a soil moisture probe (Decagon ECH_2O, Pullman, USA) calibrated for soil type and pot size. Volumetric moisture content (VMC) remained under 10% for the dry treatment and above 30% for the wet treatment. All plants were well watered just before trait measurement so that leaf trait measures would not be underestimated (Cornelissen *et al.*, 2003).

Trait Measures

Growth. At the beginning of treatment and throughout, plants were photographed once a week to estimate the growth trajectory. Using a digital camera (Nikon Coolpix P5100, Japan) on a tripod, we recorded size of individual plants by taking pictures of whole trays. We used the ImageJ (Rasband, 2011) software to measure the length of the two longest leaves of each plant. Relative growth rate was calculated by dividing the mean leaf length at the end of the experiment by the mean leaf length at the third week of treatment.

$\delta^{13}C$. We measured the carbon isotope ratio, which is a measure of integrated water-use efficiency (Farquhar & Richards, 1984). Fresh leaf material (50 mg) was collected from 1085 plants (one complete block and one half each of the two other blocks) and immediately dried in a lyophiliser for 24 hours (Edwards freeze dryer Modulyo, USA). Samples were then ground for 30 seconds with a steel bullet in a milling machine (MM300, Retsch, Germany). Samples were analyzed by isotope mass spectrometry at the University of New Hampshire Stable Isotope Laboratory (Durhman,

NH 03824, USA). Final data represent the carbon isotope ratio relative to the standard (R_{PDB}) [$\delta^{13}C$ (‰) = (R_s/R_{PDB}-1) x 1000] (Farquhar & Ehleringer, 1989). We corrected the data for the ambient ^{12}C:^{13}C ratio by subtracting the average $\delta^{13}C$ obtained from 8 corn plants, which were raised over the treatment period with the experimental plants, distributed over the three blocks. Corn utilizes C4 versus C3 metabolism, and it does not discriminate between the two carbon isotopes. Therefore, the carbon isotope ratio in its tissue is a useful reference for the ambient carbon isotope ratio.

Stomata density and stomata length. We measured stomata density and length using one fully extended and hardened leaf per plant. Using clear nail varnish, we polished a small portion of the abaxial side of the leaf and let it dry for about five minutes. Once dried, we affixed a small piece of clear tape over the polished portion. The tape was removed, leaving the dried nail polish with the stomata impressions stuck on it. The tape was then affixed to a clean microscope slide (Thermo Scientific, USA). Stomata impressions were photographed at x100 magnification using a camera attached to a microscope (Leica Microsystems GmbH Wetzlar, Germany) and analyzed using ImageJ (Rasband, 2011). Stomata density was counted over a surface of 206'822 μm^2 and then divided by dry matter (dry mass of leaf portion/surface area of leaf portion) in order to obtain stomata density per mg dry weight. We calculated stomata length as the average distance in micrometers between the guard-cell junctions of 10 stomata (Maherali *et al.*, 2002). Based on the average size of the stomata and the surrounding epidermis cells, we made sure that measurements were taken on stomata of about the same age.

Leaf dissection index. Two fully extended and hardened leaves were collected from each plant (1620 plants x 2 leaves = 3240 measures). As described before, leaves

were of the same age and had grown during the treatment period. Sampled leaves were scanned (HP Scanjet G2710) and analyzed using ImageJ (Rasband, 2011) to obtain measures of leaf perimeter and leaf area. Leaf dissection index (DI) was calculated using the Fourier transformation method described by Kincaid and Schneider (1983): $DI = perimeter/(2\sqrt{(area \times \Pi)})$. The index value is without unit but comparable to a circle with a DI of 1.

Leaf-dry-matter content. Prior to scanning, a disc was punched out from the distal end of the leaf blade, along the central vein. If a leaf was of average size, the diameter of the disc was 0.6 cm. For small leaves, we used a hole-punch of 0.3 cm diameter. The discs were immediately weighed with a precision balance (Mettler Toledo XA204DR). Then each disc was placed into a white unbleached paper bag (Flachbeutel SKB559, Germany) and dried in an oven at 60°C for 48 hours. Dry mass of each disc was then taken on a microscale (Mettler Toledo XP6, USA). Leaf-dry-matter content (LDMC) was calculated for two leaves per plant as the oven dry mass of the disc (mg) divided by its water-saturated fresh mass (g), expressed in mg g^{-1} (Cornelissen *et al.*, 2003). The averaged LDMC of the two leaves was used for the experiment.

Trichome density. We calculated trichome density by counting the total number of trichomes on the two discs of a plant and divided the number by disc area. Counting was done just before the discs were dried, directly after measuring wet weight.

Flowering time. Once plants started flowering, we recorded the day of first flower opening every 2-3 days throughout the flowering period. Prior to calculating flowering time, germination and flowering dates were adjusted to the mid-point of checking in order to be as close as possible to the true date. Flowering time was calculated as the

number of days between germination and the appearance of the first flower. Flowering time for plants that did not flower was calculated as the number of days between germination and the end of the experiment plus: 2 days for plants with big flower buds, 7 days for bolted plants with long stem, 20 days for bolted plants with short stem and 40 days for unbolted plants.

Measuring selection under drought

At the end of the experiment, plants of one northern population and a southern population (G and F, see Table 1) were kept in drought treatment for fifteen weeks. We randomly chose ten families of each population and four individuals per family, two of which had previously been in each of the two treatments. Plants were randomly split between two trays and assigned a position within tray. Plants were kept in indoor culturing facilities at 14 hours light / 22° and 10 hours dark / 18° C with a light intensity of 200-250 μmol m^{-2} s^{-1}. We gave 6 ml of water per plant every other day and recorded survival three times a week.

Statistical Analysis

G-matrix comparison. Prior to the analysis, all traits were corrected for the effect of holding tray in each treatment separately. Furthermore, in order to meet assumptions of multivariate normality, we log-transformed flowering time, relative growth and trichome density. Finally, we standardized the phenotypic trait data to a mean of zero and standard deviation of one across all populations and treatments. We estimated broad sense G-

matrices for all nine populations and in each treatment respectively by use of the following mixed-effects model:

$Y_{ijk} = \mu + F_i + I_{j(i)} + \varepsilon_{ijk}$

with the grand mean (μ) as a fixed effect and maternal family (F) and individual (I) nested within family as random effects (Dmitriew *et al.*, 2010). We estimated variance components with a Bayesian analysis and the MCMCglmm package of R v2.15.2 (Hadfield, 2010; R Development Core Team, 2012; codes in Appendix S1). We set the total number of iterations to 100'000, burn-in to 2'000 and thinning to 40. We took priors from a mixed model analysis based on restricted maximum likelihood with a moderate degree of freedom parameter (lme4 package of R; Bates *et al.*, 2012).

We compared G-matrices estimated from central populations with matrices estimated from populations of the edge of the distribution and repeated this process for each of the two treatments. We carried out several analyses comparing G-matrices. First, we calculated Kirkpatrick's total genetic variance (2009) and the eigenvalue of the first eigenvector, often defined as g_{max} (Schluter, 1996), for each G-matrix. Second, we assessed the impact of genetic correlations on constraints to adaptation comparing Kirkpatrick's effective number of dimensions (2009: eqn. 2). Third, we asked whether the size of the G-matrices (among others, given by the determinant) differed among populations by Bartlett's test (Goodnight & Schwartz, 1997; Roff *et al.*, 2012). Fourth, we calculated the angles between G-matrices estimated from central populations and G-matrices estimated from populations of range margins in two subspaces by use of Krzanowski's analysis (1979). Finally, we calculated the angle between the multivariate selection vector and the vector of predicted selection response (Lande & Arnold, 1983).

The multivariate selection vector was estimated by taking the coefficients of a linear regression with survival as response variable and all measured traits as explanatory variables. Survival data were taken from the fifteen weeks sub-drought-study. The vector of predicted selection response was estimated by multiplying each matrix to the selection vector.

We investigated the effect of the genetic cluster, treatment, latitude and the square-term of latitude (latitude^2 = latitude*latitude) on the total genetic variance, the first eigenvalue, Kirkpatrick's effective number of dimensions and the angle between the multivariate selection vector and the vector of predicted response to selection by mixed model analyses (lme4 package: Bates *et al.*, 2012). Genetic cluster, treatment, latitude and latitude^2 were predictor variables on the level of the population with population as random effect. Furthermore, significance of pairwise comparisons was unveiled by randomly re-sampling individuals of each population-by-treatment combinations, estimating G-matrices and re-calculating test statistics 200 times. G-matrices of re-sampled data were estimated by Bayesian analysis with 30'000 iterations, burn-in of 1000 and thinning of 25. Priors were taken from mixed model analysis based on restricted maximum likelihood.

Results

Substantial genetic variances and covariances among traits were present in most of the G-matrices estimated for each population-treatment combination (see Appendix S2). Principal component analysis of the G-matrices revealed that 31 to 35% of the variation

was explained by the first principal component while the second principal component explained 25 to 34% of the variation (Appendix S3).

Results of total genetic variance and first eigenvalue revealed that populations at range margins had a lower potential to respond to univariate selection on this combination of traits than central populations. First, the effect of latitude and latitude^2 on the total genetic variance was highly significant (Table 2). While a linear relationship appeared with latitude, populations of both range margins displayed lower genetic variance than in the center of the distribution (Fig. 2A). Treatment did not greatly affect the total genetic variance and only showed a tendency for lower values under dry conditions. Second, while not strongly associated to latitude and latitude^2, the first eigenvalue showed a trend for lower values at both northern and southern margins (Fig. 2B). Treatment did not affect the first eigenvalue. Overall, neither the total genetic variance nor the first eigenvalue was significantly associated with genetic cluster or the interactions between treatment and latitude or latitude^2.

Mixed model analysis revealed that the effective number of dimensions was significantly associated with latitude with decreasing values towards the northern edge (Table 2, Fig.2C). However, latitude^2 significantly affected the effective number of dimension. At the southern boundary, populations appeared to have a greater effective number of dimensions than in the distribution's center. A similar pattern was observed in the North but seem to be driven by the most northern population. The interaction between treatment and latitude or treatment and latitude2 revealed a trend for the effective number of dimensions. While more linear across latitude in the control treatment, values were

greater in the dry treatment, particularly at range margins. The effective number of dimensions was no different in either one of both genetic clusters.

Despite these observations, pairwise comparisons of test statistics demonstrated that some northern populations may be more constrained in their adaptation than central ones. While bigger in size, one northern population of the dry treatment had a smaller effective number of dimensions than central populations (Table 3 and 4). In the control treatment, the effective number of dimensions was smaller for one northern population. Southern populations did not show the same patterns: while sometimes bigger in size, the effective number dimension was neither greater nor smaller than central populations. Overall, Kirkpatrick's effective number of dimensions was generally low (between 1.60 and 2.75) indicating considerable genetic covariances between traits.

In the dry treatment, Krzanowski's comparison of subspaces revealed angles of 23° to 78° for subspace 1 but much larger (65° to 88°) for subspace 2 (Table 4). In the same treatment, comparison of subspaces between northern or southern populations of the eastern cluster with central population, revealed angles significantly departed from random. This means that G-matrices were less aligned than expected by chance. In the control treatment, angles varied between 29° and 66° for subspace 1 and between 57° and 86° for subspace 2. Angles deviated from random in the subspace 1 between one southern and one central population and in subspace 2 between one northern and one central population.

The angle between the multivariate selection vector and the vector of predicted selection response was significantly greater at the northern edge of the distribution of the control treatment than in central locations (Table 5). However, this angle was not

associated to genetic cluster, treatment, latitude or latitude^2 (Fig.2D). Pairwise comparisons of angles still imply that populations at the northern boundary may be more constrained in their adaptation than populations of central locations.

Discussion

In this study, we found evidence that populations at the northern edge of the distribution may be more limited in their adaptation compared to populations in the center. First, the total genetic variance appeared to be lower at both range margins with a similar tendency for the first eigenvalue. These results reflect a low potential to respond to selection (Kirkpatrick & Barton, 1997; Roff *et al.*, 2012) and the presence of functional constraints (Kawecki, 2008). Second, although a general overview illustrated a greater effective number of dimensions at distribution's limits, pairwise comparisons demonstrated that northern populations often displayed a smaller effective number of dimensions, reflecting the presence of strong genetic correlations and limits to adaptive evolution (Kirkpatrick, 2009). This was particularly true for the northeastern populations under drought and one northwestern population under control conditions. Moreover, comparisons of subspaces between marginal and central populations indicated that G-matrices often significantly differed. In line with these observations, northern populations displayed a greater angle between the multivariate selection vector and the vector of predicted selection response, particularly in the control treatment. Overall, treatment did not strongly affect traits integration and genetic variation across the range.

Patterns of constraints did not appear to be as strong in southern populations. While sometimes different in size, their effective number of dimensions was not

significantly different than in central locations. However, the total genetic variance and first eigenvalue appeared to be smaller than in central populations indicating a lower potential to respond to selection on this combination of traits (Kirkpatrick, 2009). Although we have good evidence that our northern populations are at the true edge of the species distribution, we also know that a few populations are present further South (Willi and Griffin, in prep). We did not include those in our study because of selfing evidence, which could similarly limit adaptation than at higher latitudes (Charlesworth & Charlesworth, 1987).

Comparisons with previous findings show that our results point in the same direction. In plant species, marginal populations often exhibit lower genetic variation than core/central populations due to none suitable habitats or mating systems (Holtken & Tahtinen, 2003; Michalski & Durka, 2007; Tsumura *et al.*, 2007). Other research based on G-matrix comparisons displayed similar results than ours. For example, Etterson (2004b) observed that northern populations of *Chamaecrista fasciculata* might be more challenged in their adaptation to climate change than central and southern population. As we indicated in our study, this limitation might be caused by trait integration and strong genetic correlation. Similar to our findings, Calsbeek and colleagues (2011) showed that the evolutionary potential of *Phalaris arundinacea* growing across large ecological gradients was driven by latitude conferring great differences in the size and shape of G between North and South. Similarly, Colautti and Barrett (2011) discovered that the invasion of *Lythrum salicaria* in northern habitats was obscured by strong correlations. In our study, the general pattern was that populations at northern margins were more

constrained in their adaptation. However, drought appeared to be a more limiting factor of the northeastern population.

Models of evolution at range margins imply that adaptation is prevented when the selective gradient steepens often leading to a diminution of genetic variation (Fisher, 1930; Wright, 1931; Kirkpatrick & Barton, 1997; Lenormand, 2002; Bridle & Vines, 2006; Sexton *et al.*, 2009). The decrease of total genetic variance at the distribution edges and measures of the angle between the multivariate selection vector and the predicted response to selection are in agreement with these models. In the control treatment, this angle appeared to be greater at the northern edge of the distribution than in central populations. These observations provide evidence of greater constrains at the northern margins. However, our measure of selection presents some limitations. The multivariate selection vector was constructed from only two populations of the studied range and therefore limits prediction for all nine populations. Moreover, our selection vector has been estimated through a regression technique (Lande & Arnold, 1983) and it has been discussed that selection coefficients are not always exactly equal to regression coefficients (only directional and correlational selection gradients are; Stinchcombe *et al.*, 2008). Still, the difference between angles found at northern boundaries offer interesting clues for the potential constraints present at in these locations.

In spite of these observations, results of dimensionality are in conflict with our earlier findings. In the dry treatment, populations of both the southern and the northern boundaries of the eastern cluster displayed larger dimensionalities, thus enclosing more genetic variance and offering evidence of a greater potential to respond to selection (Goodnight & Schwartz, 1997; Roff *et al.*, 2012). However, such observations do not

necessarily mean that populations strongly increased their evolutionary potential. With a rather small effective number of dimensions, G-matrices stay relatively "flat" (see Fig.1 of Kirkpatrick, 2009) reflecting strong genetic correlations. In this case, even with increased dimensionality, populations will remain constrained in their adaptation but will have a better ability to respond to selection in the direction of the underlying genetic correlations. Thus, the observed patterns are likely to be the results of strong genetic interactions between life history or fitness traits like flowering time and integrated water-use efficiency (Stinchcombe & Weinig, 2004; Caicedo *et al.*, 2004; Baird *et al.*, 2011; Paccard *et al*, in prep). Therefore, although the adaptive potential seems greater at northern boundaries, populations remain relatively constrained in their adaptation.

In this experiment, we produced broad-sense G-matrices and some caution has to be applied in the interpretation of our results. In this study, plants came from seeds collected in the field. Thus, our estimates could potentially be misconstrued with non-genetic effects of the maternal environment. However, in a previous study using the same data-set, we found a linear relationship between latitude and life history traits like flowering time and integrated water-use efficiency (Paccard *et al*, in prep). So far, there is almost no evidence that phenotypic differentiations across large geographic clines are the fruit of maternal effects. In our study, we know that gene flow between populations is very low (Willi & Määttänen, 2010) and as long as environmental variation is relatively large, adaptive maternal effects are not anticipated (Galloway, 2005; Montague *et al.*, 2008).

In the actual context of global changes, the rate of northern expansion in plant species is expected to slow down due to habitat fragmentation and none-available suitable

niches (Shigesada & Kawasaki, 1997; Etterson & Shaw, 2001). Therefore, their persistence at such margins is likely to depend on adaptive evolution. In this study, we have shown that populations of northern boundaries are constrained in their adaptation. This was particularly true at the northeastern margin when facing drought. As a consequence, the observed limitations are likely to play a significant role in the persistence of this species at higher latitude under future climates. More broadly, we showed the utility of G-matrix comparisons to answer and investigate patterns of constraints on adaptation. These methods are likely to be useful in the fields of ecology and conservation management.

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Table 1. List of *Arabidopsis lyrata* populations studied along a western and an eastern latitudinal cline, with information on location, genetic cluster, cline position, latitude and longitude.

Population	Location	Genetic cluster	Cline position	Latitude, N	Longitude, W
A	Fort Leonard Wood	West	southern	37° 43'	92° 03'
B	Malanaphy Springs State Preserve	West	central	43° 21'	91° 50'
C	Namekagon Barrens Wildlife Area	West	northern	46° 06'	92° 04'
D	Lake of the Woods	West	northern	49° 39'	94° 55'
E	Mayodan South	East	southern	36° 24'	79° 57'
F	Fort Hill Preserve	East	central	39° 29'	78° 55'
G	Clark Reservation	East	northern	42° 59'	76° 05'
H	Providence Forge	East	southern	37° 25'	77° 01'
I	Sandy Hook	East	central	40° 26'	73° 59'

Table 2. Results of mixed model analysis testing the effect of genetic cluster (East versus West), treatment, latitude, the square term of latitude (latitude^2) and their interaction with treatment on measures describing G matrices between populations and treatment: Total genetic variance across traits, first eigenvalue, Kirkpatrick's effective number of dimensions (Dimensions) and the angle between the multivariate selection vector and the vector of predicted response to selection (Angle). *t*-values are reported. Significant values are indicated: (*) P < 0.1, * P < 0.05, ** P < 0.01. *t*-values with *P* < 0.1 are in bold. Random effects are not shown.

	Total genetic variance	First eigenvalue	Dimensions	Angle
Cluster	0.27	-0.82	0.22	0.51
Treatment	**1.99(*)**	1.11	**-1.91(*)**	-1.08
Latitude	**4.8****	**1.78(*)**	**-2.36***	-1.36
Latitude^2	**-4.65****	**-1.71(*)**	**2.32****	1.35
Treatment*Latitude	-0.68	-1.12	**1.93(*)**	-0.66
Treatment*Latitude^2	0.69	1.14	**-1.95(*)**	0.68

Table 3. Comparisons of Kirkpatrick's effective number of dimensions between G-matrices. G-matrices estimated from populations at the edge of the distribution are compared with G-matrices estimated from populations of central locations and in both treatments respectively. Central populations are also compared together.

	Dry				Control			
Cline position	Population	Eff.Trait	Population	Eff.Trait	Population	Eff.Trait	Population	Eff.Trait
North vs Central	C	1.99	B	2.19	C	1.58	B	**2.02***
North vs Central	D	2.47	B	2.19	D	2.04	B	**2.02(*)**
North vs Central	G	1.60	F	**1.76****	G	1.83	F	2.01
North vs Central	G	1.60	I	**2.06***	G	1.83	I	2.70
Central vs Central	B	2.19	F	1.76	B	2.02	F	2.01
Central vs Central	I	2.06	F	1.76	I	2.70	F	2.01
South vs Central	A	2.54	B	2.19	A	2.03	B	2.02
South vs Central	E	2.42	F	1.76	E	2.11	F	2.01
South vs Central	H	2.75	I	2.06	H	2.45	I	2.70

Significance was revealed by re-sampling: (*) $P < 0.1$, * $P < 0.05$, ** $P < 0.01$

Table 4. Statistics of G-matrix comparison with Bartlett's test and Krzanowski's comparisons of angles between PCs. G-matrices estimated from populations at the edge of the distribution are compared with G-matrices estimated from populations of central locations and in both treatments respectively. Central populations are also compared together. Bartlett's test compares the size of the G-matrices while Krzanowski's subspace analysis tests whether the angle between the first or the second subspace between two matrices deviates from random.

		Bartlett		Krzanowski			
Populations compared	Cline positions	Dry	Control	Dry		Control	
C vs B	North vs Central	179.97	233.82	32.09°	72.73°	52.94°	**82.17°****
D vs B	North vs Central	162.08	196.7	40.78°	73.87°	29.62°	57.12°
G vs F	North vs Central	**191.08****	253.75	**55.05°(*)**	78.2°	45.68°	77.92°
G vs I	North vs Central	**241.74****	271.33	61.68°	86.63°	45.6°	64.79°
B vs F	Central vs Central	238.65	237.53	63.79°	81.1°	41.42°	76.3°
I vs F	Central vs Central	**241.85(*)**	145.69	**65.43°***	84.37°	65.17°	80.81°
A vs B	South vs Central	257.23	245.95	34.28°	65.96°	**66.69°(*)**	86.8°
E vs F	South vs Central	206.12	**277.36***	**78.9°****	88.11°	48.54°	81.47°
H vs I	South vs Central	**263.56****	139.46	23.86°	74.66°	34.29°	69.72°

Significance was revealed by re-sampling: (*) $P < 0.1$, * $P < 0.05$, ** $P < 0.01$

Table 5. Comparisons of the angle between the multivariate selection vector and the vector of predicted selection response. The multivariate selection vector was estimated from a linear regression using survival data of a fifteen weeks drought treatment period.

			Dry				Control			
Cline position			Population	Angle	Population	Angle	Population	Angle	Population	Angle
North	vs	Central	C	37.08°	B	33.66°	C	67.19°	B	**42.06°****
North	vs	Central	D	42.38°	B	33.66°	D	50.14°	B	**42.06°****
North	vs	Central	G	59.73°	F	56.97°	G	41.49°	F	**29.17°***
North	vs	Central	G	59.73°	I	33.41°	G	41.49°	I	**39.18°****
Central	vs	Central	B	33.66°	F	56.97°	B	42.06°	F	29.17°
Central	vs	Central	I	33.41°	F	56.97°	I	39.18°	F	29.17°
South	vs	Central	A	56.89°	B	33.66°	A	53.5°	B	42.06°
South	vs	Central	E	45.69°	F	56.97°	E	31.53°	F	29.17°
South	vs	Central	H	54.87°	I	33.41°	H	47.17°	I	39.18°

Significance was revealed by re-sampling: * $P < 0.05$, ** $P < 0.01$

Figure legend

Figure 1. Locations of the *Arabidopsis lyrata* populations from both a western (black triangles) and a eastern (black circles) latitudinal gradient. The two gradients depict two different ancestral genetic clusters.

Figure 2. Total genetic variance (A), first eigenvalue (B), Kirkpatrick's effective number of dimensions (C) and the angle between the multivariate selection vector and the vector of predicted response to selection (D) of each G-matrix under control (black dots) and dry treatment (grey dots) against latitude. Regression lines and quadratic curves are indicated for the two treatments when latitude or latitude^2 was significant (wet: continuous line, dry: dashed line)

Figure 1

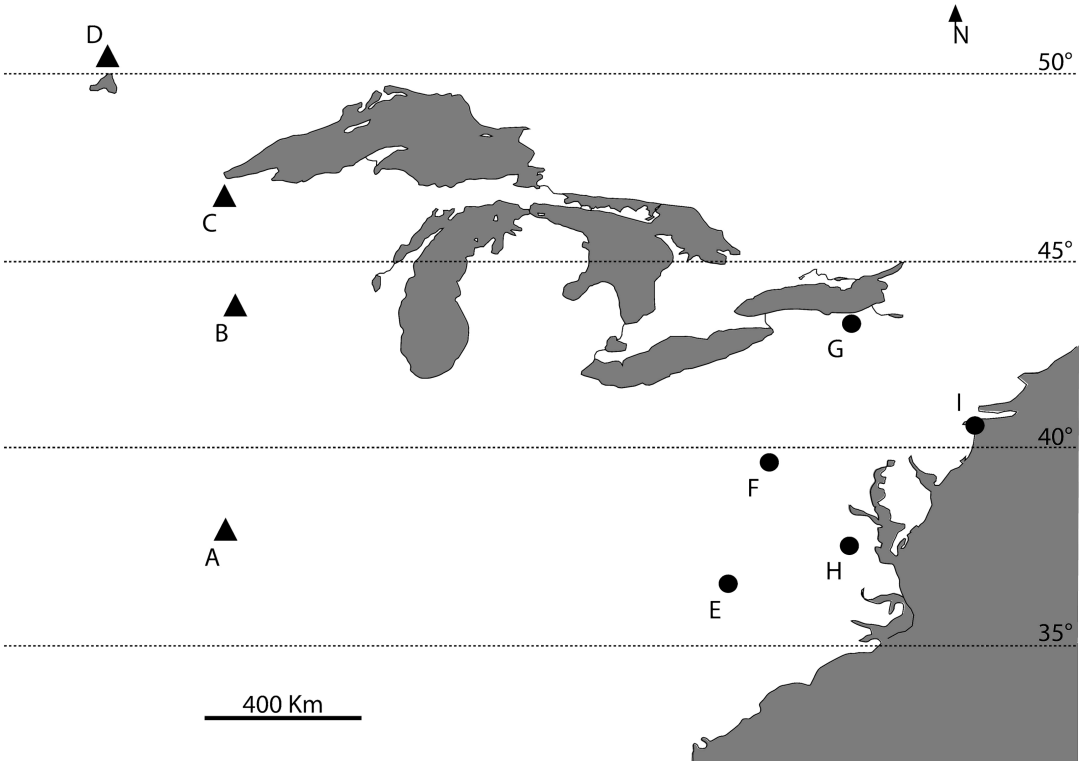
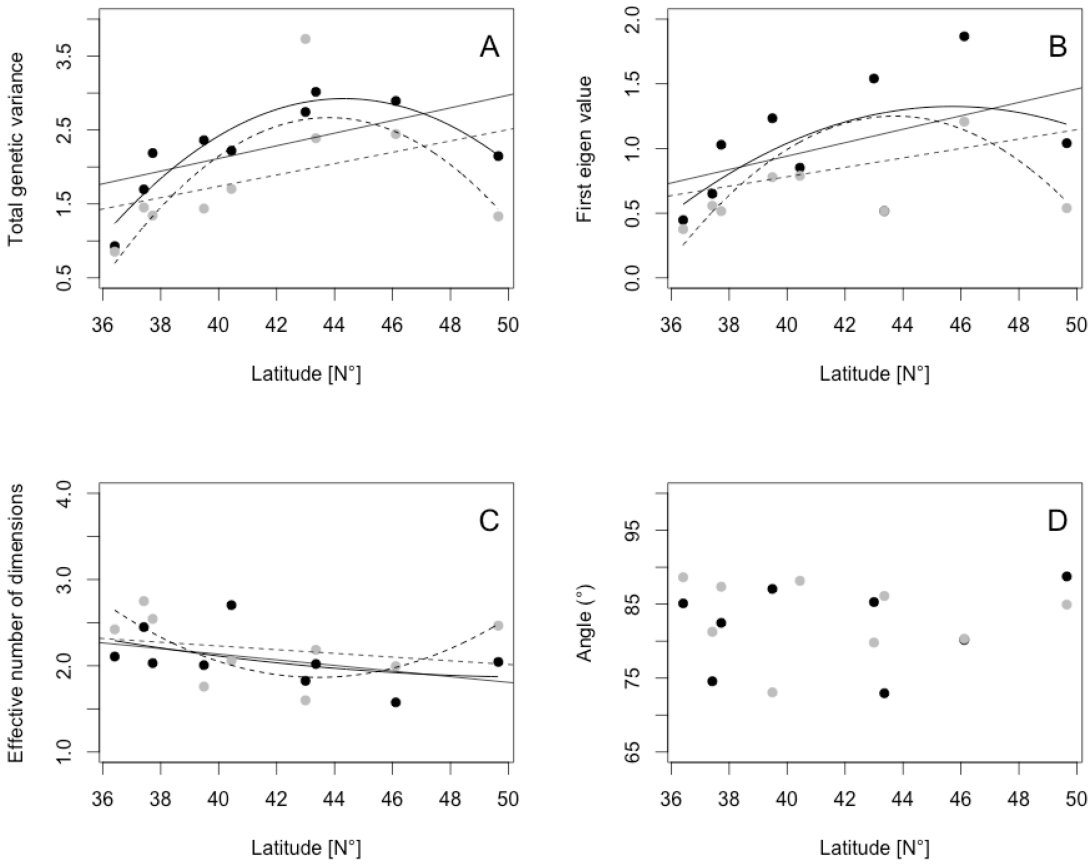


Figure 2


A
Total genetic variance
0.5
1.5
2.5
3.5
36
38
40
42
44
46
48
50
Latitude [N°]
B
First eigen value
0.0
0.5
1.0
1.5
2.0
Latitude [N°]
C
Effective number of dimensions
1.0
2.0
3.0
4.0
Latitude [N°]
D
Angle (°)
65
75
85
95
Latitude [N°]

Appendix S1. MCMCglmm codes for G-matrix estimation.

```
m.A_D.obs <- MCMCglmm(fixed = cbind(X1ft, X2di, X3growth, X4ldmc, X5stomdens,
X6stoml, X7trich, X8c13) ~ trait - 1, random = ~ us(trait):fam, rcov = ~ us(trait):units,
prior = priors, family=c("gaussian", "gaussian", "gaussian", "gaussian", "gaussian",
"gaussian", "gaussian","gaussian"), nitt=nitt.value.obs, burnin=burnin.value.obs,
thin = thin.value.obs, data=d.A_D.MCMCglmm)

VC.A_D.obs <- matrix(posterior.mode(m.A_D.obs$VCV), nrow=N_traits,
ncol=N_traits)

prior <- list(R=list(V=diag(8), n=1),G=list(G1=list(V=vc.A_D.lmer, n=4)))
```

Appendix S2. Genetic variance-covariance matrices of *Arabidopsis lyrata* of the nine populations under control and dry treatment. Estimates of the dry treatment are on the upper right side of the matrices, estimates of the control treatment are on lower left side of the matrices. The traits were flowering time (FT), leaf dissection index (DI), relative growth (RG), leaf dry-matter content (LDMC), stomata density (SD), stomata length (SL), trichome density (TD) and $\delta^{13}C$.

A.	FT	DI	RG	LDMC	SD	SL	TD	$\delta^{13}C$
FT	0.93/0.255	-0.07	0.064	0.072	0.083	-0.014	-0.084	0.096
DI	-0.032	0.169/0.162	-0.008	-0.021	-0.007	-0.011	0.08	-0.119
RG	-0.019	-0.011	0.07/0.108	-0.057	0.091	0.002	-0.003	-0.037
LDMC	0.12	-0.07	-0.026	0.188/0.267	0.023	-0.001	-0.068	0.051
SD	0.124	-0.087	-0.003	0.083	0.308/0.121	-0.055	-0.046	-0.002
SL	0.05	-0.001	0.071	-0.093	0.035	0.274/0.125	0.033	-0.006
TD	-0.019	0.062	-0.025	-0.053	-0.129	-0.067	0.155/0.103	-0.069
$\delta^{13}C$	-0.174	0.061	-0.019	-0.016	-0.047	-0.084	0.072	0.095/0.203

B.	FT	DI	RG	LDMC	SD	SL	TD	$\delta^{13}C$
FT	0.061/0.205	-0.254	-0.028	0.012	-0.02	0.008	0.002	-0.125
DI	-0.009	0.715/0.455	-0.12	0.087	-0.079	0.088	-0.027	0.189
RG	-0.038	-0.178	1.076/0.541	-0.127	0.24	-0.173	0.034	-0.14
LDMC	-0.008	0.147	-0.266	0.113/0.362	-0.179	0.217	-0.005	0.069
SD	-0.117	0.022	0.241	0.01	0.462/0.159	-0.173	-0.007	-0.056
SL	0.11	0.329	-0.12	0.064	-0.324	0.369/0.418	-0.012	0.012
TD	0.032	0.003	-0.119	-0.004	-0.082	0.067	0.048/0.032	0.011
$\delta^{13}C$	0.006	-0.031	-0.356	0.033	-0.115	0.001	0.038	0.175/0.217

C.	FT	DI	RG	LDMC	SD	SL	TD	$\delta^{13}C$
FT	0.449/0.175	-0.174	0.125	-0.002	0.03	-0.038	-0.051	0.079
DI	-0.216	0.528/0.556	-0.212	0.173	-0.199	0.102	0.029	-0.239
RG	0.108	-0.247	0.212/0.692	-0.225	0.169	-0.004	-0.014	0.001
LDMC	-0.289	0.115	-0.026	0.344/0.35	-0.235	0.061	-0.059	-0.132
SD	0.492	-0.349	0.189	-0.264	0.534/0.208	-0.057	0.027	0.087
SL	0.113	-0.177	0.108	-0.035	0.179	0.236/0.061	0.01	-0.038
TD	-0.235	0.27	-0.083	0.138	-0.371	-0.15	0.26/0.082	0.007
$\delta^{13}C$	-0.222	0.238	-0.154	0.225	-0.259	-0.05	0.057	0.331/0.321

D.	FT	DI	RG	LDMC	SD	SL	TD	$\delta^{13}C$
FT	0.353/0.242	-0.058	0.092	0.098	0.036	0.039	0.031	-0.152
DI	-0.047	0.276/0.147	-0.039	-0.027	-0.045	0.012	-0.02	0.056
RG	-0.056	0.094	0.196/0.111	0.019	0.027	0.014	0.008	-0.125
LDMC	0.041	-0.057	-0.043	0.037/0.216	0.058	-0.09	0.038	-0.049
SD	0.126	-0.05	0.165	0.014	0.474/0.096	-0.106	0	-0.001
SL	-0.306	0.059	-0.012	-0.012	-0.346	0.491/0.237	-0.007	-0.044
TD	0.008	-0.076	-0.088	0.016	-0.057	0.057	0.097/0.031	-0.018
$\delta^{13}C$	0.145	-0.167	-0.108	0.06	0.053	-0.091	0.055	0.223/0.252

E.	FT	DI	RG	LDMC	SD	SL	TD	$\delta^{13}C$
FT	0.155/0.089	0.033	-0.052	0.056	-0.024	-0.019	-0.037	0.082
DI	0.081	0.103/0.053	-0.046	0.041	0.003	0.007	-0.014	0.033
RG	-0.023	0.019	0.098/0.118	-0.044	-0.032	0.014	0.05	-0.081
LDMC	0.001	0.021	0.029	0.111/0.14	-0.06	0.003	-0.042	0.049
SD	0.054	-0.004	0.002	-0.048	0.147/0.127	-0.054	0.005	0.002
SL	0.079	0.064	0.034	0.034	0.013	0.105/0.137	0.022	-0.076
TD	-0.063	-0.04	0.016	-0.047	-0.005	-0.054	0.078/0.043	-0.065
$\delta^{13}C$	-0.117	-0.071	-0.007	-0.058	-0.007	-0.092	0.092	0.132/0.147

F.	FT	DI	RG	LDMC	SD	SL	TD	$\delta^{13}C$
FT	0.177/0.074	0.003	0.055	0.001	-0.001	0.005	-0.054	-0.016
DI	-0.067	0.137/0.107	0.03	-0.013	0.073	-0.033	-0.176	-0.092
RG	0.128	-0.031	0.176/0.212	0.095	0.089	-0.045	-0.128	-0.031
LDMC	-0.059	0.096	-0.051	0.167/0.133	0.09	-0.036	-0.017	0.005
SD	0.066	-0.095	0.061	-0.009	0.323/0.25	-0.061	-0.206	-0.1
SL	0.024	-0.03	0.044	-0.159	-0.189	0.501/0.102	0.028	0.018
TD	0.166	-0.074	0.1	-0.112	-0.122	0.382	0.672/0.427	0.167
$\delta^{13}C$	-0.087	0.106	-0.052	0.135	0.056	-0.165	-0.248	0.21/0.13

G.	FT	DI	RG	LDMC	SD	SL	TD	$\delta^{13}C$
FT	0.796/1.18	-0.102	0.415	0.016	0.297	0.601	-0.485	-0.164
DI	-0.167	0.349/0.094	-0.117	0.021	-0.133	-0.123	0.07	0.074
RG	0.106	0.069	0.129/0.647	-0.011	0.052	0.286	-0.335	-0.19
LDMC	0.038	0.098	-0.017	0.204/0.168	-0.1	-0.013	-0.109	-0.011
SD	0.082	-0.096	-0.002	0.049	0.125/0.407	0.273	-0.126	-0.059
SL	-0.097	0.175	0.048	-0.02	-0.095	0.205/0.606	-0.364	-0.14
TD	-0.536	0.27	-0.043	0.171	-0.097	0.169	0.746/0.499	0.147
$\delta^{13}C$	0.153	-0.101	-0.023	-0.052	0.005	-0.057	-0.235	0.19/0.131

H.	FT	DI	RG	LDMC	SD	SL	TD	$\delta^{13}C$
FT	0.187/0.382	-0.132	0.054	0.031	-0.02	-0.065	-0.024	0.09
DI	-0.026	0.247/0.194	-0.015	-0.002	-0.11	0.094	0.011	-0.034
RG	-0.019	-0.081	0.148/0.059	-0.053	-0.033	0.032	0.004	-0.03
LDMC	0.004	0.126	-0.006	0.184/0.218	0.005	-0.075	0.045	0.077
SD	0.061	-0.075	0.004	-0.003	0.192/0.156	-0.085	-0.049	0.01
SL	-0.005	-0.054	0.055	-0.008	0.031	0.06/0.151	0.019	-0.057
TD	-0.136	0.054	-0.095	-0.003	-0.027	-0.025	0.347/0.055	-0.07
$\delta^{13}C$	-0.163	0.007	0.064	0.029	-0.108	-0.006	0.155	0.331/0.236

I.	FT	DI	RG	LDMC	SD	SL	TD	$\delta^{13}C$
FT	0.194/0.385	0.009	-0.025	0.002	0.008	-0.108	-0.045	0.102
DI	0.029	0.101/0.073	-0.04	0.019	-0.035	0.012	-0.095	0.038
RG	0.127	-0.003	0.764/0.251	-0.065	0.082	-0.038	0.136	-0.069
LDMC	-0.045	-0.015	0.067	0.162/0.066	-0.048	0.01	-0.086	0.074
SD	-0.03	-0.047	-0.007	-0.105	0.188/0.101	0.004	0.18	-0.126
SL	0.08	0.063	0.119	-0.03	-0.063	0.207/0.093	0.013	-0.076
TD	-0.221	-0.061	0.034	0.119	0.017	-0.124	0.445/0.364	-0.205
$\delta^{13}C$	-0.113	0.005	-0.056	0.102	-0.043	-0.031	0.155	0.16/0.368

Appendix S3. PCA on G-matrices estimated by MCMCglmm for each population-treatment combination. The eigenvectors of the first two principal components are reported. The bottom line reports % variance explained by each principal component.

	A-dry		B-dry		C-dry		D-dry	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Flowering time	0.45	0.07	-0.19	0.63	0.35	-0.44	0.41	-0.24
Dissection index	-0.41	0.20	0.35	-0.43	-0.42	0.13	-0.39	0.01
Relative growth	0.10	0.60	-0.41	-0.17	0.32	0.00	0.38	-0.29
Leaf-dry matter	0.28	-0.35	0.37	0.29	-0.38	-0.35	0.36	0.32
Stomata density	0.32	0.49	-0.41	-0.27	0.41	0.23	0.29	0.48
Stomata length	-0.24	-0.35	0.37	0.33	-0.40	0.02	-0.11	-0.64
Trichme density	-0.48	0.07	-0.31	0.03	-0.01	0.78	0.40	0.05
$\delta^{13}C$	0.39	-0.32	0.36	-0.35	0.35	0.00	-0.38	0.34
% total variance	0.33	0.31	0.35	0.31	0.33	0.25	0.34	0.30

	E-dry		F-dry		G-dry		H-dry	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Flowering time	0.42	0.10	0.26	-0.38	0.38	0.06	-0.31	0.43
Dissection index	0.39	0.11	0.38	-0.28	-0.38	0.23	0.42	-0.28
Relative growth	-0.40	0.12	0.38	0.15	0.37	0.15	0.17	0.65
Leaf-dry matter	0.34	0.39	0.28	0.63	0.03	0.83	-0.27	-0.49
Stomata density	-0.03	-0.70	0.40	0.09	0.33	-0.41	-0.39	-0.06
Stomata length	-0.22	0.54	-0.31	-0.47	0.40	-0.02	0.48	0.09
Trichme density	-0.43	0.01	-0.40	0.25	-0.39	-0.23	0.31	-0.24
$\delta^{13}C$	0.41	-0.16	-0.38	0.27	-0.39	-0.10	-0.39	-0.05
% total variance	0.33	0.27	0.35	0.31	0.33	0.25	0.34	0.29

	I-dry		A-control		B-control		C-control	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Flowering time	0.19	0.61	0.32	-0.17	0.33	-0.52	0.36	-0.34
Dissection index	0.38	-0.19	-0.42	0.09	0.25	0.31	-0.36	-0.36
Relative growth	-0.36	0.22	0.09	0.61	-0.38	-0.33	0.34	0.40
Leaf-dry matter	0.41	-0.17	0.32	-0.47	0.34	0.50	-0.34	0.58
Stomata density	-0.42	0.11	0.44	-0.09	-0.38	0.39	0.37	-0.12
Stomata length	-0.15	-0.69	0.20	0.58	0.36	-0.23	0.34	0.44
Trichme density	-0.42	0.05	-0.43	-0.14	0.41	-0.12	-0.36	-0.07
$\delta^{13}C$	0.39	0.17	-0.44	-0.11	0.36	0.23	-0.35	0.21
% total variance	0.34	0.28	0.33	0.28	0.35	0.33	0.35	0.31

	D-control	E-control	F-control	G-control

	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Flowering time	0.35	0.32	0.40	-0.29	0.37	0.30	-0.40	0.11
Dissection index	-0.43	0.07	0.42	-0.01	-0.35	-0.36	0.41	0.17
Relative growth	-0.31	0.40	0.03	0.54	0.34	0.33	-0.14	0.62
Leaf-dry matter	0.46	-0.08	0.29	0.49	-0.41	0.00	0.23	-0.52
Stomata density	0.14	0.53	0.04	-0.62	-0.12	0.67	-0.35	-0.39
Stomata length	-0.26	-0.48	0.43	0.02	0.34	-0.40	0.38	0.37
Trichme density	0.26	-0.46	-0.44	0.02	0.39	-0.24	0.42	-0.14
$\delta^{13}C$	0.47	0.01	-0.44	0.03	-0.42	0.10	-0.40	0.08
% total variance	0.34	0.29	0.31	0.25	0.34	0.30	0.34	0.30

	H-control		I-control	
	PC1	PC2	PC1	PC2
Flowering time	0.35	-0.44	0.45	0.03
Dissection index	-0.40	-0.34	0.33	0.33
Relative growth	0.28	0.40	0.18	0.11
Leaf-dry matter	-0.26	-0.35	-0.31	0.49
Stomata density	0.40	-0.19	-0.05	-0.71
Stomata length	0.40	0.31	0.42	0.25
Trichme density	-0.38	0.22	-0.45	0.03
$\delta^{13}C$	-0.32	0.48	-0.43	0.26
% total variance	0.35	0.34	0.33	0.28

General conclusion

This thesis focused on answering three important questions in evolutionary biology by use of quantitative genetics and multivariate statistics. The three questions were: the role of habitat heterogeneity on genetic variation within populations, the traits of latitudinal adaptation and their underlying genetics, and the factors limiting range expansion at the northern and southern edge of species distribution. All questions were addressed in the study species of *Arabidopsis lyrata* of North America. With water availability being one of the most critical environmental characteristics for plants, this thesis specifically focused on drought responses.

The one trait that was found to show presumably adaptive differences across a dune-gradient within population and across latitude among populations was flowering time. In **Chapter 1**, plants of a heterogeneous sand dune landscape significantly differed in flowering time. In the well-watered treatment, plants of dune bottoms flowered about five weeks earlier than those of dune tops. This finding offers strong evidence of microhabitat adaptation and documents that a heterogeneous environment can lead to strong trait differentiation, which could play a significant role in the maintenance of genetic variation within populations. In **Chapter 2**, plants stemming from northern latitudes with lower precipitation during the vegetation period flowered earlier and showed a tendency for greater drought adaptation than plants from southern latitudes. Furthermore, lower watering led to earlier flowering, which highlighted the presence of co-gradient variation. Such findings are still rare in plant species and indicate the presence of past adaptive evolution in this trait. One evolutionary outcome of co-gradient variation is the promotion of range expansion: the establishment of individuals beyond their range margins would be facilitated by trait values that are impacted similarly by both the new environment and genetics.

When using a multivariate approach for investigating patterns of genetic variation, differences within and among populations were found beyond the single trait of flowering time. In **Chapter 1**, genetic variation measured as the number of independent trait dimensions was larger in the whole population than in each of the two microhabitats of dune tops and bottom, at least under control conditions. This result was only partly driven by flowering time as the most important contributors of the first eigenvector of the G-matrix estimated from the dune-bottom microhabitat under control conditions were stomata density and length, but not flowering time. This means that while habitat heterogeneity might facilitate adaptation by loosening overall trait integration, this may be driven by several traits that seem developmentally and/or physiologically integrated. In **Chapter 3**, multivariate analysis demonstrated that populations at the edge of the distribution had lower levels of genetic variation than those in the centre of the distribution. Additionally, stronger patterns of further potential evolutionary constraints were found for two northern populations compared to central populations: the number of trait dimensions was smaller and the angle between the multivariate selection vector and the predicted response to selection was significantly greater. Flowering time was only one of the traits that contributed to genetic integration, others were at least as or more important, but their contribution varied among populations and treatment. These results

clearly show that evolutionary studies focusing on the evolution of individual traits are important, but that many traits with no apparent link to focal traits influence the trajectory of evolution and should therefore be considered as well.

Results of this thesis did not always support predictions of past theoretical work. For example, the role of habitat heterogeneity had long been defined as one of the major factors driving the maintenance of genetic variance within populations. However, empirical studies had revealed conflicting results and heterogeneous habitats did not necessarily increase levels of genetic variance. **Chapter 1** took a novel approach by multivariate statistics and found that such heterogeneity can slightly increase the adaptive potential but not via an increase of genetic variation, but by relaxing genetic constraints. This result suggests that the maintenance of genetic variation probably includes properties of the G-matrix and changes in interactions of quantitative traits. In contrast, results of **Chapter 3** were in line with predictions made by theory. As anticipated by evolutionary models, a significant decrease in the amount of quantitative genetic variation was present in marginal habitats.

In summary, while major progress was made in quantitative genetics over the last two decades, findings of **Chapters 1** and **3** highlight the importance of multivariate statistics in answering important ecological and evolutionary questions. Evolution does not act on single phenotypes or loci, and processes of adaptation should be investigated across many traits. Unfortunately, the lack of theory focusing on potential constraints built upon trait integration makes it difficult to generate empirical studies for testing theoretical predictions. Future work should go in that direction.

Most plant species are dependent on their propensity to flower and to deal with water content. Consequently, results of this thesis are transferable to many organisms. However, while similar patterns of constraints could be present at range margins, a species' capacity to adapt to local conditions will depend on their ability to rapidly shift trait values. This will depend on both plasticity and the amount of genetic variation present within populations. In this thesis, substantial plasticity in response to drought was present for most of the traits. Accordingly, several herbaceous species are likely to respond in a similar way and the present results offer strong clues on the capacity of such plants to deal with drought conditions.

In the context of global climate change, results of this thesis have very practical implications for the fields of applied ecology and conservation biology. First, adaptive evolutionary responses require genetically based variation among individuals. Therefore understanding how levels of genetic variance are maintained in populations is crucial for predicting species persistence under environmental changes. This thesis proposes that the habitat heterogeneity can be an important factor of evolutionary potential. Second, discerning past adaptation to environmental conditions helps anticipating the traits likely to be under selection under future environmental change. Estimates on levels of genetic variation in these traits can be used to predict longer-term population persistence. Finally, the response to environmental change may be constrained by the genetic integration of traits that are not in accordance with the direction of selection. As a result, selection is likely to be inhibited by the presence of strong genetic correlations. Therefore, it appears

essential to discern constraints in species adaptation so that better conservation strategies can be constructed. In particular, understanding limits to adaptation is crucial for predicting rates of extinction imputable to climate change as well as infestation risks by invasive species, future crop yields and – as a more down-stream effect – the functioning of ecosystems.

Overall, this thesis contributed to the advancement of understanding the capacity of populations to cope with past and future environmental change. This work has shown that progress in the field necessitates improved knowledge in the following areas: (1) We need additional theory at the multivariate level so that testable predictions can be constructed for empirical research. (2) The properties of G-matrices need to be better understood for the investigation of patterns of constraints and maintenance of genetic variation. (3) More empirical data is needed about gene flow between core and margins of species distributions and the type and strength of selection at range margins to estimate their role in limiting range expansion. (4) Large- and small-scale transplant experiments should be executed to reveal the traits of adaptation and the genetic constraints met at range margins. Theoretical and empirical development of these issues will greatly advance our understanding of the limits of adaptation