Climate mal-adaptation and biotic interactions at species' range limits

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Abstract

Why species have restricted geographic distributions or why species do not occur everywhere is still an open question in ecology and evolutionary biology. It is assumed that the species range limits normally reflect the ecological conditions where the species stop occurring because of a lack of habitat suitability. Moreover, these populations at the margins are known to suffer from a history of small population size and the accumulation of genetic drift. Additionally, biotic interactions have been recently proposed to act negatively at the range edges. Among them, pollination services are particularly important as reproduction and population dynamics of the majority of the flowering plant species rely upon them. However, pollinator services are not constant, varying across different temporal and spatial scales.

Here I tested whether a history of small population size, enhanced genetic drift, and the accumulation of deleterious mutations in range-edge populations was linked with reduced adaptation in the North American *Arabidopsis lyrata*. I performed a transplant experiment with sites across and beyond the species distribution with source plant populations from the centre and the periphery, these last ones with a history of range expansion or long-term isolation. Additionally, I monitored pollination interactions in natural populations over a transect spanning from the southern to the northern range limit and over different temporal and spatial scales using time-lapse cameras.

The results from the transplant experiment shown that plant multiplicative performance declined toward the southern range limit and beyond, but not in the northern range. Furthermore, populations shown evidence of climate adaptation to two suggested niche variables, temperature in spring, and precipitation of the wettest quarter. However, the signature of adaptation was reduced in populations with a history of small population size, and additionally, the heterosis effect was increased in populations with heightened genomic

estimates of load, longer expansion distance or long-term isolation, and a selfing mating system. Genetic drift and mutation accumulation due to past range expansion and long-term isolation of small populations at the range margins is therefore a strong determinant of population-mean performance.

In the pollinators project, I found that the plant-pollinator network for *A. lyrata* is a generalist system, and southern populations had lower pollination services compared to center and northern populations. The diurnal activity of the pollinators was mostly explained by air temperature conditions, occurring the majority of the visits during the mid-day. The density of flowers in a patch explained partially the spatial variation, but the signature was specific for each taxonomic group. Even though no evidence of niche partitioning was found, the different taxonomic groups of pollinators differed in their activity window where some taxa were more tolerant under certain temperatures or intervals of the day.

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General introduction

An open question in evolutionary ecology is why species are limited in their geographic distribution and what the main processes are that constrain distribution (Hoffmann & Blows 1994; Kawecki 2008; Sexton *et al.* 2009; Willi & Buskirk 2019). There is a large volume of literature showing that physiological tolerance and resistance to abiotic factors strongly affect the distribution of species (Hargreaves *et al.* 2014; Lee-Yaw *et al.* 2016). Less attention has been paid to the consequences of small population size and inbreeding caused by past range expansion or rear-edge isolation. Range edge populations might be constrained not only because of low habitat suitability but also because of enhanced genetic drift, reducing genetic diversity required for adaptation under a changing environment and opposing the effect of selection. Apart from climate and genetic factors, additional factors may influence the species range, including biotic interactions. This includes all the interactions between species that can affect their performance and distribution, such as competition, parasites, and pathogens, herbivory, or pollination services. Pollination services are particularly relevant for flowering plants as most of them rely on animal pollinators for reproduction (Ashman *et al*. 2004). The mutualistic interaction might be impoverished at range limits which could feedback to reduce plant population size and the success to colonize new environments. However, pollination services are far from constant and pollinator activity relies on abiotic factors and specific preferences for flower display, which additionally contribute to the specifics of the pollination network. Species distribution limits might therefore be a result of a complex interplay between intrinsic and extrinsic factors, that have not been explored and disentangled so far.

In accordance with Hutchinson's (1957) ecological niche theory, range limits reflect abiotic and biotic conditions under which a species cannot maintain self-sustaining populations. In other words, a reduction in habitat suitability from the centre to the edges of a species' range might be responsible for the decrease in population mean fitness at the

margins, impeding the expansion of the species (Gaston 2003). Inline, centre populations are predicted to have a higher density of individuals than peripheral populations (abundant-centre hypothesis; Brown 1984). If the performance of the organism decreases at the range edge and beyond, we can assume that range limits are the spatial representation of niche limits (Sexton *et al.* 2009 Hargreaves *et al*. 2014; Lee-Yaw *et al*. 2016). Marginal populations affected by reduced habitat suitability might suffer from long-term small population size and isolation, or they may have a history of being small because of past range expansion (Willi & Buskirk 2019). The predicted decay in population size toward range edges implies an increase in genetic drift. One of its predicted consequences is the reduction in genetic variation required to adapt to novel habitats or to fluctuating environments (Nei *et al.* 1975; Eckert *et al*. 2008). Furthermore, genetic drift is expected to oppose directional selection (Kimura *et al.* 1963), leading to reduced local adaptation. Both, extrinsic factors related to habitat quality and intrinsic factors linked to a history of genetic drift and reduced local adaptation, might lead to the stabilization of range limits. How adaptation at the range edge and beyond affects lifetime fitness in these small and isolated marginal populations facing extreme or more fluctuating ecological conditions has not been completely understood.

Another aspect to consider when studying range limits and the causes that potentially affect populations to expand or contract is related to the accumulation of deleterious mutations (reviewed in Willi & Buskirk 2019). Small population size at range edges, caused either by reduced habitat suitability or past range expansion, should enhance genetic drift and reduce the efficacy of purifying selection. The predicted consequence is the accumulation of deleterious mutations that reduce population mean fitness toward range edges (Henry *et al*. 2015; Peischl *et al.* 2015). The accumulation of mutational load at range edges or along routes of range expansion has recently been documented in humans (Henn *et al.* 2016), and in plants

(Willi *et al*. 2018; Koski *et al*. 2019). However, the contribution of mutational load to range limits has not been assessed in nature.

In addition to abiotic and intrinsic genetic factors, biotic interactions may be an important piece of the puzzle of species range limits. The importance of biotic interactions has not been considered in species distribution models (SDM), nor their potential in shaping the geographic ranges of species. However, several field studies showed their likely involvement in range limits, including interspecific competition (Stanton-Geddes *et al*. 2012), parasites and pathogens (Briers 2003; Coates *et al*. 2017), herbivory (Benning *et al*. 2019), and flower-insect interactions (Chalcoff *et al*. 2012; Moeller *et al*. 2012). As most plants depend on pollinators for pollen transfer and reproduction, plant population dynamics are likely to vary with pollination activity and pollinator diversity. Pollination services may for example be limited at the range edge if i) plant population densities are too low to attract visitors (Elliott & Irwin, 2009); if ii) changes in floral display are accompanied by a lower attractiveness (Dart *et al*. 2012); if iii) the plant species richness is low to provide diverse floral resources to the pollinators (Biesmeijer *et al*. 2006); or if iv) the ecological conditions are not ideal for pollinator activity (Herrera 1990). As pollinator activities can vary with abiotic factors and are far from constant, their role in establishing the range edges or driving adaptation remains uncertain.

Even though the activity of pollinators might be explained by abiotic ecological conditions (Herrera 1990), physiological tolerances and behavioural preferences might also explain their abundance and variation in space and time (Gilbert 1985). Tolerances and preferences might be species-specific and often vary among pollinator groups, possibly in part to avoid resource competition (Stone *et al*. 1999). This temporal and spatial variation in pollination services implies additional challenges to fully understand the constitution and

resilience of the interaction network and to predict the consequences of species loss on the side of plants and pollinators.

In my thesis, I addressed in a plant species whether range limits coincide with niche limits and whether marginal populations with a history of genetic drift bear a signature of reduced climate adaptation (**Chapter I**). I performed a crossing experiment combined with a field transplant experiment to evaluate the effect of mutational load and heterosis in population performance in the field (**Chapter II**). I assessed pollination services across the geographic distribution of the plant species, comparing visitation rates between the core and the margins, and investigated the mechanistic processes explaining visitation rates (**Chapter III**). Finally, I studied variation in pollination on diverse scales of time and space (**Chapter IV**).

Study system

To investigate range limits, patterns of climate adaptation, a history of genetic drift and mutational accumulation, and the role of biotic factors in marginal population, I worked with the model organism *Arabidopsis lyrata* subsp. *lyrata. Arabidopsis lyrata* (lyre-leaved rock cress) is a close relative of the plant model species in genetics, *A. thaliana*. The two species share a divergence time estimated to six million years (Hohmann *et al.* 2015). However, the species differ in mating system and chromosome number. While *A. thaliana* is self-compatible and diploid with 5 pairs of chromosomes, *A. lyrata* is mostly self-incompatible and diploid with 8 pairs of chromosomes; but tetraploid populations of *A. lyrata* in Europe and self-compatible populations in North America are known (Koch & Kiefer 2005; Griffin & Willi 2014). The difference in the number of chromosomes between species does not surprise as in the Brassicaceae family, as they are known to vary widely, from n=4 in *Physaria* to n=128 in *Cardamine concatenate* (Koch & Kiefer 2005).

The *Arabidopsis lyrata* species has a circumpolar arctic-alpine distribution and consists of two subspecies: *A. lyrata* subsp. *petraea*, in central and northern Eurasia, and *A. lyrata* subsp. *lyrata*, in North America (Schmickl *et al.* 2010). The North American *A. lyrata* forms two distinct ancestral clusters. The eastern cluster is found from North Carolina to Upstate New York, and the western cluster in the Midwest, from Missouri to south-western Ontario (Willi & Määttänen 2010; Griffin & Willi 2014; Willi *et al.* 2018). The natural range limits of the (sub-) species and the evolutionary consequences of population marginality is the focus of this study.

The species is a short-lived perennial plant that forms basal rosettes, and blooms in early spring, with 10mm long white flower (Fig. 1). The flowers are known to produce nectar discs at the base of the anthers and volatile compounds to attract pollinators (Peer & Murphy 2003). Flowers are visited by many pollinator species, varying depending on the population, but they are mostly visited by bees, Syrphids, and Bombyliids. The habitat where the species can be found is on sand dunes and rocky outcrops, as well as on sandy or rocky riverbanks and shorelines.

Figure 1. An image of the study organism, *Arabidopsis lyrata*, growing on bare rock (left), and flowers visited by *Callophrys grynaeus* (right).

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Chapter 1: **Reduced climate adaptation at range edges in North American *Arabidopsis lyrata***

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Abstract

Species range limits, when not caused by dispersal limitation, reflect constraints in the evolution of the ecological niche. Here we tested whether a history of small size and enhanced genetic drift of range-edge populations was linked with reduced adaptation. We performed a transplant experiment with sites across and beyond the species distribution of North American *Arabidopsis lyrata*, with plants from the centre of distribution, and the periphery with a history of range expansion or long-term isolation. Performance declined toward the southern range limit and beyond, suggesting that southern range limits – but not northern ones – reflected niche limits. Furthermore, we found adaptation to two important niche- and range-determining environmental variables, temperature in spring and precipitation during the wettest quarter. However, the signature of adaptation to precipitation was reduced in populations with a history of small population size. Therefore, we conclude that reduced adaptation is a contributor to range limits.

Impact Summary

Evolutionary theory on species' ranges along linear environmental gradients suggests that genetic drift due to small population size can constrain adaptive evolution and cause abrupt range limits. We tested the role of genetic drift in adaptation across the distribution of *Arabidopsis lyrata*. We performed a large-scale transplant experiment with sites within and beyond the species range, and with seed material from populations of the core and periphery of distribution, with the latter sharing a history of genetic drift. Our results showed that range limits reflected limits to the ecological niche of the species in the south, as transplanted populations were mostly non-persistent beyond the southern range limit. However, range limits in the north did not reflect niche limits, which may be a consequence of recent climate warming. Furthermore, evidence for climate adaptation across populations was found, but the magnitude of adaptation was reduced in populations with a more pronounced history of genetic drift typical for range edge populations. Our results support that adaptation at geographic range edges is constrained due to genetic drift, but not the alternative mechanism of gene swamping. As genetic drift is enhanced at many species range limits, we think that reduced adaptation is a problem at many range limits.

Introduction

Several hypotheses have been put forward for why species are limited in their geographic distribution, but so far, it is unclear what the main constraining processes are (Hoffmann & Blows 1994; Kawecki 2008; Sexton *et al.* 2009; Willi & Van Buskirk 2019). If dispersal limitation is found not to be relevant at species range limits, geographic distributions reflect niche limits (Chown & Gaston 1999; Hargreaves *et al.* 2014). The evolutionary explanation for range limits then is that populations at the edges fail to adapt and expand their ecological niche. Evolutionary models have suggested the conditions under which adaptation at range edges fails and which therefore cause range limits (reviewed in Sexton *et al.* 2009). Here we focused on the fact that many species show enhanced signatures of genetic drift toward range edges (Pironon *et al.* 2017), which may be linked with the reduced potential to adapt. The lack of adaptation to range-edge conditions due to genetic drift may prevent the further spread of the species into more extreme environments and be one of the causes of range limits. Here, we tested the hypothesis that adaptation at current range edges is reduced and that this is connected with a history of long-term small size.

The role of increasing genetic drift towards range limits on adaptation has not been explored conclusively by evolutionary theory on species ranges. One set of models tracks adaptation and range expansion by assuming a linear gradient of environmental change and a polygenic trait under selection. Adaptation is predicted to lead to the expansion of the range unless dispersal is long and the environmental gradient steep, which leads to maladaptation, the gradual decline in population mean fitness, and eventually to range limits (Kirkpatrick & Barton 1997). When the action of both selection and genetic drift are considered, the same sort of model predicts that range limits establish by two contributors: steep environmental gradients, and either genetic drift opposing selection or genetic drift eroding genetic variation (Polechová & Barton 2015; Polechová 2018). Two aspects are noteworthy. Dispersal has a mixed effect; it

increases dispersal load but lowers the magnitude of genetic drift. Furthermore, population sizes and genetic drift are fairly constant across the range. A second set of models works with source-sink dynamics and addresses whether a sink site that differs in ecological conditions can be occupied and adapted to (reviewed in Kawecki 2008). Here the prediction is that adaptation and persistence in the sink is more likely if gene flow is not too restricted because it brings in recruits and genetic variation important for local adaptation (Holt & Gomulkiewicz 1997; Holt *et al*. 2003). While some of the source-sink models included the action of genetic drift, its role in the source, which could stand for the outermost edge-population, was not explored.

There are several reasons why range edges may commonly have a history of small population size that then affects the potential to adapt via genetic drift opposing selection or eroding genetic variation. First, based on empirical observations, a purely ecological hypothesis was formulated, namely that species have high abundance in the range centre and lower abundance at the range periphery because of a decline in habitat quality or habitat availability (abundant-centre hypothesis; Hengeveld & Haeck 1982; Brown 1984). A recent meta-study provided strong support for this hypothesis as 81% of studies were found to report a significant decline in population occurrence from the centre to the periphery (Pironon *et al.* 2017). In principle, this should lead to the enhanced exposure to genetic drift, as was suggested by the population-genetic extension of the abundant-centre hypothesis (Eckert *et al*. 2008). A completely different hypothesis advocates that during range expansion, serial demographic bottlenecks accompanied by genetic drift leave a pattern of declining genetic diversity from the area of expansion start toward the expansion end (Excoffier *et al*. 2009). Indeed, many species underwent relatively recent range expansion due to Pleistocene glaciation cycles, which left an imprint of small population size toward range edges (Hewitt 1996; Hewitt 2000). In support of both hypotheses outlined above, Pironon *et al*. (2017) found an overall significant decline in genetic marker diversity from the centre to the periphery across species ranges, with 47% of

studies showing a significant decline. Therefore, we can conclude that many edges of species ranges, have a history of small size and heightened exposure to genetic drift, either due to less available habitat or past range expansion.

This motivates the question of whether range edge populations with a history of increased genetic drift are less adapted to local environmental conditions. The testing for local adaptation is best done with a reciprocal transplant experiment (Kawecki & Ebert 2004). By performing a general transplant experiment across a species distribution and beyond, we asked whether range limits coincide with niche limits (I), whether populations were adapted to the local climate (II), and whether range edge populations with low genetic diversity were less adapted (III). Study organism was the short-lived perennial plant *Arabidopsis lyrata* subsp. *lyrata* from North America. A previous niche-modelling study on *A. lyrata* indicated that northern and southern range limits coincided with niche limits, with minimum temperature in early spring and precipitation during the wettest quarter being the variables that predicted species occurrence best (Lee-Yaw *et al.* 2018). Furthermore, populations toward range edges were shown to have a history of small population size due to postglacial range expansion and rear-edge isolation, and mating system shifts to selfing associated with range edges (Griffin & Willi 2014; Willi *et al.* 2018). In line, higher genetic diversity and therefore larger effective population sizes were found in areas from which colonization started, which nowadays are near to the geographic centre of distribution (Willi *et al.* 2018). The twenty populations involved in the study represented the geographic centre of distribution as well as the peripheries.

Material and methods

Study organism and within-population crosses

North American *A. lyrata* subsp. *lyrata* (from now on abbreviated as *A. lyrata*) is distributed along the eastern US, from North Carolina to Upstate New York, and in the Midwest, from Missouri to south-western Ontario, forming two distinct ancestral clusters (Willi & Määttänen 2010; Griffin & Willi 2014; Willi *et al.* 2018). It is mostly a self-incompatible, insect-pollinated plant that produces basal rosettes. However, a fraction of self-compatible and selfing populations were found at the edges of species distribution (Griffin & Willi 2014). The species is found on sand dunes and rocky outcrops, as well as on sandy or rocky riverbanks and shorelines.

Twenty populations of *A. lyrata* were selected because they represented the total distribution in North America from south to north, two ancestral genetic clusters and both mating systems (Fig. 1, Table S1). For each natural population, in 2007, 2011, and 2014, mature fruits of 30-50 plants were collected over a surface area of about 450 m^2. To reduce the effects of the site of origin and to get a high number of seeds of known genotypic composition, we raised plants indoors to perform within-population crosses. For each population, two seeds of 26 seed families were sown in pots and later thinned to one plant per pot (see Table S2 for raising conditions). Plants of each population were randomly assigned to be either mother plants/dams receiving pollen (12), father plants/sires being pollen donors (12), and backup plants (2). Each dam was randomly assigned a sire from the same population. Hand pollinations were performed on emasculated flowers at the bud stage. The crossing was repeated until 6-7 fruits or about 60 seeds were available per cross combination. The experiment resulted in 224 crosses with seeds for sowing in the transplant experiment.

Transplant experiment

Five transplant sites were established along a latitudinal gradient in the eastern US (Fig. 1). Sites were selected based on the position relative to the species range: beyond the northern edge, in the Adirondacks, NY (CG1); near the northern range edge, in Williamstown, MA (CG2); in the centre of distribution, in Harrisonburg, VA (CG3); near the southern range edge, in Winston-Salem, NC (CG4); and, beyond the southern range edge, in Athens, GA (CG5), (Fig. 1, Table S3). The start of transplanting at the sites was adjusted to the local climate, about 6 weeks before the long-term daily average temperature fell to 10 °C. The setup started in August 2017 for the site beyond the northern edge (CG1) and ended two months later at the southernmost site (CG5). At the southern range edge (CG4), sowing had to be repeated in December of the same year because of chloride in the water.

At each transplant site, three replicate pots with two seeds each were prepared per cross combination. The three pots per cross were then split into three spatial blocks, and within the block, they were randomly assigned to 13 multi-pot trays with 38 pots each (note that not all pots were filled with seeds analyzed here; others contained between-population crosses; see Perrier *et al.* 2020). Pots had a diameter of 7 cm, a depth of 6 cm, were perforated at the bottom, and filled with a 1.5:1 mix of unfertilized peat moss and washed sand. The same protocol was followed at all sites. As some crosses had produced only a few seeds (cross combinations with less than 30 seeds), we replaced them with another maternal line of the same population, or only one seed was sown per pot. A total of 7,098 seeds were sown (5 transplant sites x 20 populations x 12 maternal lines x 3 blocks x 2 seeds per pot – 102 missing seeds, Table S4).

Pots were immediately placed outdoors, into a meadow, under a portable walk-in greenhouse to keep conditions favorable for germination for the first 10-12 days; an exception was the transplant site at the southern range edge where the second round of germination occurred inside the university glasshouse. When the portable greenhouse was removed, a white

mesh cloth protected seedlings from being washed away for another week. Plants were watered as needed, keeping the soil surface moist during the first month to promote germination. Later on, plants were exposed to the natural local conditions at each site. However, competitive interactions were avoided by removing other plant species and covering the surrounding area with a black foil. Herbivore pressure was partially controlled: with a fence around the blocks, ant traps against seed predation in the first fall, and organic slug repellent in the first spring. When in the same pot two seedlings germinated, one was haphazardly removed.

Plant performance was tracked weekly or more regularly, starting with the sowing of seeds in late summer 2017 until the end of the reproductive season in June 2019 (for a list and description of traits see Table S5). *Reproductive output* was assessed in each of the two reproductive seasons, 9 weeks after the first few plants flowered at a site in year 2 (2018), and 5 weeks after the start of flowering in year 3 (2019). It was the total number of fruits, pedicels (flowers that did not produce a fruit but contributed with pollen), flowers, and buds. Finally, *multiplicative performance* (*MP*) was calculated as g*ermination rate* observed in a pot times *reproductive output* up to year 3. At the end of the experiment, *root length* was measured as the distance from the centre of the rosette to the end of the longest root. Unfortunately, the transplant in the centre (CG3) had to be removed in fall 2018 because the site was needed for another experiment; to compare across sites, we therefore performed also analyses on *MP* up to year 2. Between fall 2018 and spring 2019, we additionally performed a seed-burial experiment to study seed survival. Seeds of multiple maternal plants of a population were pooled, packed in bags, and left on the soil surface in every common garden (Fig. S1).

Climate data

Analysis of climate adaptation focused on the two most niche- and range-determining climatic variables, minimum temperature in early spring and precipitation during the wettest quarter

(Lee-Yaw *et al.* 2018). This data of the sites of origin of populations, and – for precipitation – for the transplant sites, was extracted from WorldClim database version 2.0 (Fick & Hijmans 2017). Temperature data at the transplant sites was collected by loggers in each garden. Five of them per site were installed 1.5 m above the ground, close to the pots and in shade, and recorded at an interval of 1 h. The difference between WorldClim-based minimum temperature in early spring, in March and April, at the site of origin of a population and the corresponding temperature measured with loggers at a transplant site was calculated and abbreviated with Δ_{Temp}. The difference between WorldClim-based precipitation during the wettest quarter at the site of origin of a population and a transplant site was abbreviated with Δ_{Prec}. The testing for local adaptation was based on absolute values, $|\Delta_{Temp}|$ and $|\Delta_{Prec}|$, with estimates close to zero indicating little difference in conditions between those populations had experienced at their site of origin and those experienced at a transplant site.

Statistical analysis

All main analyses were performed on *multiplicative performance* as the dependent variable. A first generalised linear mixed model (GLMM) tested whether range limits coincide with niche limits (research question I). The analysis was performed in a Bayesian framework (MCMCglmm in R; Hadfield 2009; R Core Team 2019) because *MP* was 0-inflated and required the analysis of both the logistic part with the 0s and the Gaussian part of the distribution (values $\log_{10}$-transformed if >0). Fixed effect was common garden, with the reference garden in the centre of the range (CG3). Random effects were block nested within transplant site, population, and family nested within population. MCMCglmm analysis was run on 10 parallel chains, with a burnin of 5000, thining of 100, and a nitt-value of 200,000. To assess whether the species had self-persistent populations within the range but not beyond the range limits, we estimated the growth rate of each population at each transplant site by creating stage-classified

matrices (Caswell 2001; see Fig. S1). Furthermore, life stages were tested for their contribution to performance in the common gardens. *Germination* and *survival* were estimated as binary variables (0, 1). *Survival* $_{\text{year 1}}$ took into account the germination state (i.e. NA if the seed did not germinate; 0 if the plant died before the end of winter in 2017/18; 1 if it survived); and *survival* $_{\text{year 2}}$ was based on *survival* in year 1 (i.e. NA if the plant had died before). *Damage* on the rosette or on the inflorescence was also treated as binary. *Time to flowering*, the *severity of the damage* (1: 0-25%; 2: 26-50%; 3: 51-75%; 4: 76-100%), *reproductive output*, and *root length* were continuous variables. All these variables were analysed individually with restricted maximum likelihood, with the R package lme4 (Bates *et al.* 2015) and lmerTest (Kuznetsova *et al.* 2017). Fixed and random effects were the same as specified above.

In the second part of the analyses, tests addressed whether populations showed a signature of climate adaptation (research question II) and whether that signature depended on the history of genetic drift (III). The main dependent variable was again *multiplicative performance*, analysed by a GLMM and Bayesian statistics. Fixed effects were $|\Delta_{\text{Temp}}|$, $|\Delta_{\text{Prec}}|$, genomic diversity, and the interaction between the former two variables and genomic diversity. Genomic diversity was assumed to reflect long-term population size, and species-wide estimates were shown to be well explained by expansion distance or rear-edge isolation, mating system and ancestral cluster (74% of variation explained; Willi *et al.* 2018). The estimate of genomic diversity was Tajima's π of intergenic regions revealed by pool-sequence analyses of population samples (Willi *et al.* 2018, Table S1). Random effects were common garden, block nested within common garden, population, and family nested within population. Secondary analyses focused on the role of the same fixed effects on life stage variables.

Results

Environmental conditions

Climate differed strongly between the five transplant sites (Table S3). Minimum temperature in early spring increased gradually from north to south, while precipitation during the wettest quarter increased from the centre toward beyond the range edges. Mean annual temperature at each transplant site was slightly warmer than expected based on longer-term averages depicted by the WorldClim data set (Table S3). The trend was strongest for the central and northern common garden sites.

I. Do range limits reflect niche limits?

A first main model tested for the effect of the position of common gardens across the *A. lyrata* distribution on *multiplicative performance* up to year 3 (Table 1; mean values of common gardens in Table S6). The effect of the common garden was significant for the site in the north, south, and beyond the southern edge in the logistic part of the model, depicting whether plants made it to flowering. Values were significantly higher at the northern edge, and lower at the southern edge and beyond the southern edge compared to the common garden in the centre of distribution, CG3. For the log-normal part of the model, depicting the number of flowers produced, only the common garden at the northern edge differed, with lower values compared to CG3. Figure 2A combines results of the two parts of distribution, illustrating low overall *multiplicative performance* at the gardens at the southern edge and beyond the southern edge of distribution, but little difference between common gardens in the north. In line, the population growth rate, *r*, was much reduced and median values across populations around 0 at the southern edge and beyond the southern edge, indicating that populations are mostly non-persistent when transplanted beyond the southern edge (Fig. 2B, Tables S6, S7). Growth rates at the northern sites were not significantly different from those in CG3 and overall on the

positive side. Results indicate that southern range limits reflect niche limits, while northern range limits do not seem to represent the species niche limits.

Further analyses focused on the effect of the common garden on life stage components as summarized in Table S7. *Germination* was significantly lower in the north and beyond the northern edge, but significantly increased at the southern edge (there seeds were raised in a greenhouse). In the first year, *survival* was significantly higher in the northern sites and at the southern edge, compared to CG3, while *survival* $_{\text{year 2}}$ was significantly lower in all common gardens compared to CG3, and strongest in the south and at the northern range edge. *Time to flowering* in year 2 was not significantly different at the northern edge and beyond the northern edge but significantly longer at the southern edge and beyond the southern edge, indicating that plants in the south flowered later relative to when soil temperature increased above 5°C (or after snowmelt). The *reproductive output* to year 2 was significantly lower at both southern sites, but also in the north, and here in year 2 and when the output of year 2 and year 3 were added (Table S7). Roots were longer at the southern sites (not measured at CG3 and therefore comparison made with CG1). Finally, *damage to rosettes* was more common in the north and beyond the northern edge, but *damage severity* was lower compared to CG3. *Damage severity* on rosettes was also reduced at southern sites. Overall, these results supported the much reduced performance and population growth rate in the southern-most transplant site mainly due to reduced overall longevity of plants.

II. *Are populations adapted to the climate conditions of their site of origin?*

III. *Is the effect of adaptation reduced in populations of range edges with low genetic diversity and a history of stronger genetic drift?*

The main model on *multiplicative performance* revealed adaptation to both temperature and precipitation (Table 2). The absolute difference in minimum temperature in early spring

between population origin and transplant site, $|\Delta_{Temp}|$, had a negative effect on the logistic process of performance, with fewer non-zero values the larger the difference was (Fig. 3A). In other words, plants had a higher chances to succeed from the seed stage to reproduction under more similar temperature conditions between home and transplant site, indicating temperature adaptation. A trend was already observed up to year 2, and the correlation became significant up to year 3. The absolute difference in precipitation during the wettest quarter between the site of population origin and transplant site, $|\Delta_{Prec}|$, had also a negative effect but this time on the log-normal part of the distribution of *multiplicative performance*. Once plants reproduced successfully, a greater performance was observed under similar precipitation as at the site of origin, indicating adaptation to the precipitation regime. Tajima's π did not affect the plant performance, however, there was an interaction with the ecological predictor of $|\Delta_{Prec}|$, with an effect on the normal part of the distribution for *multiplicative performance*. Once plants succeeded with germination and achieved reproduction, they revealed a signature of stronger climate adaptation the higher genomic diversity was and the weaker the history of genetic drift characteristic of marginal populations was (Fig. 3B).

Analyses on life-stage variables showed when patterns of adaptation emerged (Table S8). Adaptation to temperature was already expressed at the life stage of germination. *Germination* was reduced the more different the temperature regime was between the site of origin and site of assessment. Other life stages that contributed as a trend were *reproductive output* to year 3 and *root length*. *Reproductive output* tended to be lower and the roots shorter the more different the temperature regime was between site of origin and site of assessment. Adaptation to precipitation was expressed in later stages, by a decrease in *survival* $_{\text{year 2}}$, while the damage of inflorescences decreased under more different precipitation conditions population experienced. However, there was no particular life stage when the interaction between Tajima's π and $|\Delta_{Prec}|$ was significant.

Discussion

I. Do range limits reflect niche limits?

Our study attempted to first answer the question of whether range limits are the spatial representation of niche limits by combining results from species distribution modelling and transplant experiments. For *A. lyrata*, Lee-Yaw *et al.* (2018) had found that southern and even more so northern range limits were predicted well by habitat suitability, mainly defined by average minimum temperature in early spring, and precipitation of the wettest quarter. Results of the transplant experiment across the distribution and beyond confirmed that range limits reflect niche limits in the south. At the southern edge and beyond, plant performance was significantly lower compared to the centre of distribution and growth rates were around 0 at the southern edge and beyond the southern edge (Fig. 2). However, the northern range edge may not be a reflection of niche limits anymore. At the northern edge and beyond, plant performance seemed comparable with that at the centre of distribution and growth rates were not significantly different. Overall, for the southern range edge of *A. lyrata*, there is good agreement with meta-analyses showing that range limits often equal niche limits (Hargreaves *et al.* 2014; Lee-Yaw *et al.* 2016).

However, secondary analysis performed on life stage variables showed that conditions in the north were not systematically better for *A. lyrata*. Germination was lower at northern sites. Then subsequent survival ($survival_{\text{year 1}}$) was initially higher at the northern sites but then changed to lower during the second year. Furthermore, the total reproductive output to year 2 was greater at the centre and was lower at least at the northern edge. Plants in the north seemed to be more affected by herbivores, while the severity of the damage was higher at the centre site. These results provide partial support that also northern conditions may be constraining for the species and to some extent limiting. We also found that the climatic conditions during the

study were on average warmer than the long-term average based on WorldClim data, with strongest increase in the centre and at the northern two sites, which may have made especially northern sites more benign for *A. lyrata* than they used to be. While the prevailing conditions may have been unusually warm during our study, the trend is also the one observed with climate warming. Therefore, we hypothesize that northern range limits may not reflect niche limits any more for *A. lyrata* due to global warming, but that the species is nowadays dispersal limited at the cold end of distribution. Such a result was found for example for 38% of non-forest plant species of the European Alps (Rumpf *et al.* 2019).

II. Are populations adapted to the climate conditions of their home site?

Adaptation to local conditions is a common finding of transplant studies (Leimu & Fischer 2008; Hereford 2009). Furthermore, local adaptation may most often be due to climate or other abiotic factors but to a lesser extent due to biotic interactions (Hargreaves et al. 2020). Here we found evidence for adaptation to minimum temperature in early spring, the most niche- and range-determining variable formerly revealed by distribution modelling, and precipitation during the wettest quarter; plant performance was better when conditions at transplant sites were similar to those at the site of origin. These signatures of adaptation measured on plant multiplicative performance were detected in the logistic part of the distribution for temperature, with more zeros when conditions were different than those at the sites of origin. Adaptation to precipitation was detected in the normal part of the distribution of multiplicative performance, determined by the total number of reproductive organs produced once plants reached the flowering stage. Similar results were found in the analysis of life stages. Adaptation to temperature was expressed early in life, mainly during germination (with additional trends for reproductive organs and root length). In contrast adaptation to precipitation was revealed later in life, in survival of the second year. This difference in timing of expression suggests that

adaptation to temperature may involve fewer selection targets already expressed during germination, while adaptation to precipitation may be due to more genetic variants of small phenotypic contribution with cumulative effect only visible later in life. Evidence for local adaptation to temperature has been numerous in plants, e.g., shown in transplant experiments performed across latitude (e.g., Ågren & Schemske 2012), or across elevation (Halbritter *et al.* 2018). Furthermore, it may not be uncommon that local adaptation to temperature is expressed at an early life phase, as also in *A. thaliana* adaptation to southern versus northern conditions strongly involved the seedling establishment phase (Postma & Ågren 2016).

III. Is the effect of adaptation reduced in populations of range edges with low genetic diversity and a history of stronger genetic drift?

A main result of this study is that adaptation to the second important niche- and range-determining variable was dependent on the history of genetic drift experienced by populations. Results pointed to long-term small populations having a relatively low fitness peak when the precipitation regime was similar between the site of origin and site of assessment, but that this fitness peak was higher and wider for populations with long-term large size, coming from the centre of the distribution. In this sense, populations of large size may be more tolerant of a wider range of precipitation regimes (Fig. 3B). In *A. lyrata*, populations with low genomic diversity and therefore a history of small size and genetic drift generally have a history of either postglacial range expansion or long-term rear-edge isolation. The pattern was found for outcrossing populations, and the handful of selfing populations detected at range edges were confirmed to have an even more pronounced pattern of reduced genetic variation (Willi *et al.* 2018). The result of a reduced signature of adaptation in populations with a history of stronger genetic drift are in line with a result from European *A. lyrata*. A transplant experiment over an elevational gradient revealed local adaptation in one pair of low-/high-elevation populations

with higher genetic diversity, but no signature of such adaptation in another pair of populations with lower genetic diversity (Hämälä *et al.* 2018). Higher tolerance to extreme environmental conditions in populations of range cores compared to peripheral populations was found in a *Triticum* species. Plants of the core of distribution and range edges were exposed to experimental conditions found beyond the range edge, and core populations coped better with those (Volis *et al.* 2014). In our study, life-stage analyses did not reveal a particular timing of when genetic drift inferred with local adaptation or the evolution of tolerance, suggesting that its expression is due to many genetic variants each of small effect, which are more prone to be affected by genetic drift.

Range limits have recently been suggested to be a result of a failure of local adaptation due to genetic drift opposing selection or genetic drift eroding genetic variation (Polechová & Barton 2015; Polechová 2018). Our study supports that local adaptation is constrained by genetic drift associated with range-edge position. In line, Vergeer & Kunin (2013) found in a transplant experiment with sites and populations from the core and periphery of European *A. lyrata* that plant performance was generally higher in the core of distribution. Furthermore, populations from the southern range edge with the smallest census sizes were the least locally adapted. In *Plantago major*, a transplant experiment including a site in the core of distribution and two toward the northern edge revealed local adaptation of both core and edge populations, but the extent of local adaptation in edge populations with lower genetic diversity tended to be lower (Halbritter *et al.* 2015). In *A. lyrata*, the likely mechanism for this reduced adaptation is that genetic drift opposes selection. The alternative, that genetic drift impedes adaptation via a loss of genetic variation seems less likely. A quantitative genetics experiment involving populations from the centre and edges of distribution of *A. lyrata* showed that genetic variation for ecologically relevant traits was not much reduced in range-edge populations, and genetic

correlations among them were weaker, which overall produced a pattern of similar adaptive potential (Paccard *et al.* 2016).

Our study reinforces the idea that populations at range margins with a history of strong genetic drift, caused by past range expansion, rear-edge isolation, or a selfing mating system, have a reduced signature of adaptation and lower tolerance of atypical environmental conditions. This puts them in a position of a lower population mean performance due to maladaptation on the one hand and makes them weak colonizers on the other hand. Apart from their higher mutational load by genetic drift opposing purifying selection (Perrier *et al*. 2020), narrow and low adaptation to climate may together be main causes of geographic species distribution limits.

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Author contributions

All authors designed the study. YW collected seeds in the field. DSC and AP performed the crossing and transplant experiments. DSC analyzed the data. DSC wrote the first draft of the manuscript, and YW contributed to revisions

Data accessibility statement

All data is stored in Dryad (https://doi.org/10.5061/dryad.cc2fqz642)

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Table 1. Summary of model testing for the effect of common garden on multiplicative performance (*MP*) in a transplant experiment of *Arabidopsis lyrata*

		Fixed effects, logistic process										
		CG1 (Beyond north)		**CG2 (North, edge)**			**CG4 (South, edge)**			**CG5 (Beyond south)**		
Dependent variable	N	Mean	HPD	Mean	HPD		Mean	HPD		Mean	HPD	
MP $_{\text{to year 3}}$	1950	-0.154	(-0.821,0.365)	**0.599**	(-0.000,1.165)	*	**-0.978**	(-1.578,-0.428)	**	**-1.499**	(-2.136,-0.941)	***
MP $_{\text{to year 2}}$	1950	-0.215	(-0.838,0.358)	0.545	(-0.035,0.137)	(*)	**-1.055**	(-1.637,-0.497)	**	**-1.556**	(-2.149,-0.951)	***
		Fixed effects, log-normal process										
		CG1 (Beyond north)		**CG2 (North, edge)**			**CG4 (South, edge)**			**CG5 (Beyond south)**		
Dependent variable	N	Mean	HPD	Mean	HPD		Mean	HPD		Mean	HPD	
MP $_{\text{to year 3}}$	1950	0.061	(-0.126,0.278)	**-0.522**	(-0.730,-0.283)	***	0.176	(-0.069,0.412)		0.103	(-0.241,0.0.392)	
MP $_{\text{to year 2}}$	1950	-0.034	(-0.237,0.164)	**-0.532**	(-0.746,-0.317)	***	-0.256	(-0.498,0.019)	(*)	-0.085	(-0.450,0.218)	

The effect of each common garden is compared with the one in the centre of distribution (CG3). Multiplicative performance ($\log_{10}$-transformed if >0) followed a Gaussian distribution with 0-inflation. Therefore, models assessed all fixed and random effects for their importance in both the logistic process (binary variable depicting germination, survival and capacity to initiate flowering; prediction of non-zeros) and the Gaussian process (total number of reproductive organs). Estimates of coefficients are modes of an MCMC sample from the posterior distribution of parameters (*mean* and higher posterior density, *HPD*, interval). Estimates with *P*-values < 0.05 are written in bold; significance is indicated: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Results for random effects are not shown.

Table 2. Summary of model testing for the effect of the absolute difference in temperature between site of origin of populations and transplant site, $|\Delta_{Temp}|$, the absolute difference in precipitation, $|\Delta_{Prec}|$, genomic diversity depicted by Tajima's π and interactions on multiplicative performance (*MP*) in a transplant experiment of *Arabidopsis lyrata*

		Fixed effects, logistic process										
		$\|\Delta_{Temp}\|$			$\|\Delta_{Prec}\|$		**Tajima's π**		$\|\Delta_{Temp}\|*\pi$		$\|\Delta_{Prec}\|*\pi$	
Dependent variable	***N***	Mean	HPD		Mean	HPD	Mean	HPD	Mean	HPD	Mean	HPD
MP to year 3	1950	**-0.042**	**(-0.076,-0.004)**	*	-0.002	(-0.007,0.004)	0.064	(-0.063,0.193)	-0.004	(-0.013,0.007)	0.0001	(-0.0014,0.0016)
MP to year 2	1950	-0.035	(-0.075,-0.003)	(*)	-0.001	(-0.007,0.005)	0.081	(-0.047,0.216)	-0.006	(-0.017,0.004)	0.0002	(-0.0013,0.0018)
		Fixed effects, log-normal process										
		$\|\Delta_{Temp}\|$			$\|\Delta_{Prec}\|$		**Tajima's π**		$\|\Delta_{Temp}\|*\pi$		$\|\Delta_{Prec}\|*\pi$	
Dependent variable	***N***	Mean	HPD		Mean	HPD	Mean	HPD	Mean	HPD	Mean	HPD
MP to year 3	1950	-0.020	(-0.047,0.008)		**-0.005**	**(-0.010,-0.001)**	*	0.016	(-0.065,0.099)	0.002	(-0.005,0.010)	**0.001**	**(0.0002,0.0025)**	*
MP to year 2	1950	0.007	(-0.019,0.035)		**-0.005**	**(-0.009,-0.000)**	*	0.038	(-0.047,0.113)	-0.004	(-0.011,0.003)	**0.001**	**(0.0002,0.0026)**	*

Multiplicative performance (log_{10}-transformed if >0) followed a Gaussian distribution with 0-inflation. Therefore, models assessed all fixed and random effects for their importance in both the logistic process (binary variable depicting germination, survival and capacity to initiate flowering; prediction of non-zeros) and the Gaussian process (total number of reproductive organs). Estimates of coefficients are modes of an MCMC sample from the posterior distribution of parameters (*mean* and higher posterior density, *HPD*, interval). Estimates with *P*-values < 0.05 are written in bold; significance is indicated: (*) $P<0.1$, * $P<0.05$. Results for random effects are not shown.

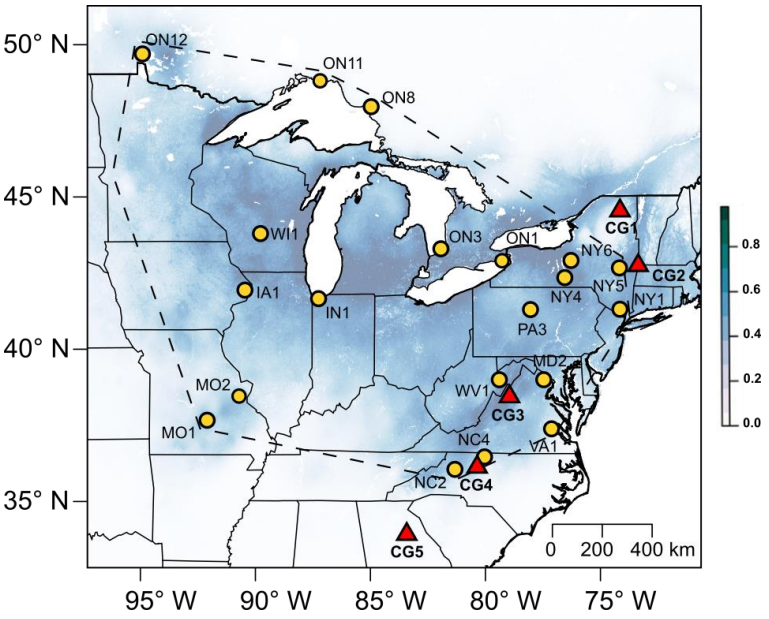

Figure 1. **Distribution of the 20 selected *Arabidopsis lyrata* populations and the location of the five common gardens (CG) transplant sites in North America**. Orange dots accompanied by a three-digit abbreviation represent the sites of origin of populations (Table S1, the two letters stand for the state in the US or the province in Canada, the number for latitudinal position within state, or longitudinal position within province as in Willi *et al.* 2018). Red triangles represent the location of each transplant site, followed by a number in sequence of initial sowing. The dashed line is the minimum convex polygon connecting the outermost populations of west and east. Shades of blue indicate habitat suitability revealed by niche-modelling (Lee-Yaw *et al.* 2018).

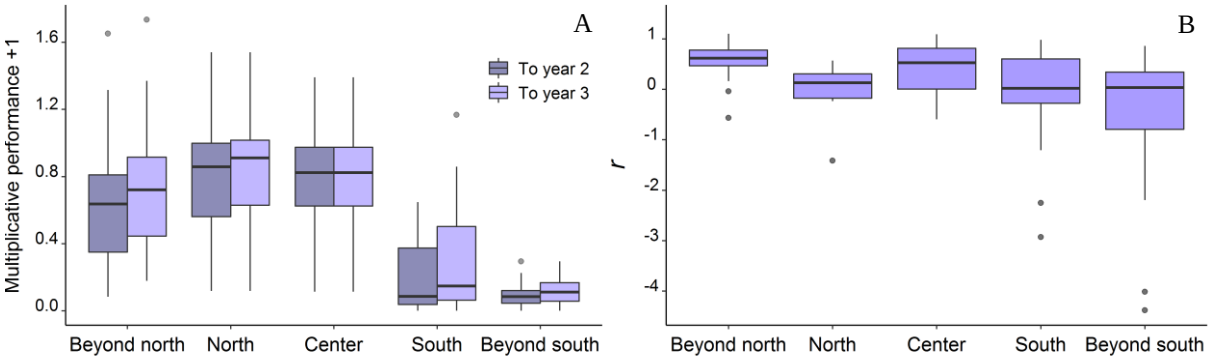

Figure 2. Multiplicative performance (A) and population growth rate (B) of *Arabidopsis lyrata* differing between transplant sites, sorted from north (left of the x-axis) to south (right). Panel A shows box plots based on population mean multiplicative performance up to year 2 or year 3. Population means were based on family means of pot-level multiplicative performance that was first log-transformed. Panel B shows box plots of population growth rate, *r*, of *Arabidopsis lyrata* populations at the five transplant sites, again sorted from north to south. The thick line of each box plot represents the median, the coloured box represents the interquartile range, the whiskers represent the variability outside the upper and lower quartiles, and individual dots represent the outliers.

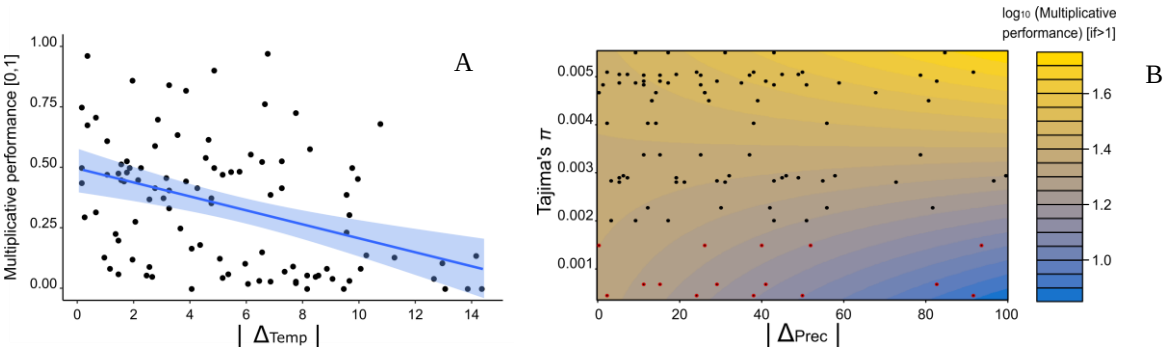

Figure 3. Relationship between multiplicative performance up to year 3 and absolute difference in temperature (A) and in precipitation, and genomic diversity, in *Arabidopsis lyrata* (B). In panel A, the population mean logistic response of multiplicative performance (0 or 1) is plotted against the absolute difference in minimum temperature in early spring between site of origin of populations and transplant site, $|\Delta_{Temp}|$ in °C. The model-predicted regression line is shown in blue, with the lower and upper 95% confidence interval. Panel B is a contour plot representing the predicted relationship between multiplicative performance up to year 3 (if values >0, in shades from blue to yellow) and both, the absolute difference in precipitation of the wettest quarter between site of origin of populations and transplant site, $|\Delta_{Prec}|$ in mm, and genomic diversity, Tajima's π. In both panels, dots are population means based on family means, for each common garden. Red dots represent selfing populations located at range edges, black dots the outcrossing populations.

Supporting information

Table S1. Ecological and genomic information on the 20 *Arabidopsis lyrata* populations studied

Population	State/Province	Latitude [° N]	Longitude [° W]	Position	T_{min}espr [°C] †	PrecWQ [mm] †	Growing season †	Tajima's π ‡	Mating system ‡
IA1	Iowa	41.97	90.37	Center	-0.9	319	7	0.0040	Outcrossing
IN1	Indiana	41.61	87.19	South	0.0	296	7	0.0034	Outcrossing
MD2	Maryland	38.99	77.25	Center	3.8	290	9	0.0055	Outcrossing
MO1	Missouri	37.72	92.06	South	4.5	324	9	0.0020	Outcrossing
MO2	Missouri	38.47	90.71	South	4.0	292	9	0.0006	Selfing
NC2	North Carolina	36.04	81.16	South	3.8	363	9	0.0022	Outcrossing
NC4	North Carolina	36.41	79.96	South	5.0	326	9	0.0029	Outcrossing
NY1	New York	41.30	73.98	Center	0.4	326	8	0.0051	Outcrossing
NY4	New York	42.35	76.39	North	-2.5	283	7	0.0051	Outcrossing
NY5	New York	42.66	74.02	North	-3.0	296	7	0.0051	Outcrossing
NY6	New York	42.99	76.09	North	-2.1	292	7	0.0049	Outcrossing
ON1	Ontario	42.87	79.18	Center	-1.7	281	7	0.0014	Selfing
ON11	Ontario	48.77	87.13	North	-7.9	283	6	0.0004	Selfing
ON12	Ontario	49.65	94.92	North	-7.8	275	6	0.0029	Outcrossing
ON3	Ontario	43.26	81.84	Center	-2.6	278	7	0.0028	Outcrossing
ON8	Ontario	47.93	84.85	North	-7.5	302	6	0.0028	Outcrossing
PA3	Pensylvania	41.28	77.87	Center	-1.4	316	7	0.0049	Outcrossing
VA1	Virginia	37.42	77.02	South	5.5	332	10	0.0049	Outcrossing
WI1	Wisconsin	43.83	89.72	Center	-3.3	307	7	0.0047	Outcrossing
WV1	West Virginia	38.96	79.29	Center	1.1	294	9	0.0045	Outcrossing

The list reports: the name of the populations used in the experiment; the state (US) or province (CAN); coordinates; the position within the distribution area of *A. lyrata*; the average minimum temperature in early spring (T_{min}espr); precipitation of the wettest quarter (PrecWQ); length of the growing season, defined as the number of months with a mean temperature higher than 5 °C; Tajima's π of intergenic regions; the mating system. Data extracted from WorldClim (†). Genomic data from Willi et al. (2018) (‡).

Table S2. Conditions at each stage of the crossing experiment of *A. lyrata* for seed propagation

Process	Location	Temp. daytime [°C]	Temp. nighttime [°C]	Day length [h]	Light intensity [µmol m^{-2} s^{-1}]	Relative humidity [%]	Duration
Stratification	Cold room	-	4	0	0	0	12 days
Germination	Growth chambers *	20	20	8	150	50	22 days
Plant growth †	Growth chambers *	22	20	16	240	50	46 days
Crossing	University glasshouse	22	20	16	240	50	6 moths
Storage of siliques	Cold room	-	4	0	0	0	1-3 months

* CLF Plant Climatics, Wertingen, Germany

† Day length and light intensity were gradually increased every three days by 1 h and 20 µmol m^{-2} s^{-1}, respectively.

Table S3. Ecological information on transplant sites

Transplant site	Location	Position	Lat. [° N] Long. [° W]	Dist. to pop [km]	Starting date	T_{min}espr [°C]	T_{mean}annual [°C]	T_{mean}annual [°C] †	PrecWQ [mm]	Snow cover [days]	Growing season
CG1	Adirondacks (NY)	Beyond northern edge	43.96 74.22	120	11.08.2017	-5.8	6.3	4.4	333	173	6
CG2	Williamstown (MA)	Northern edge	42.71 73.20	8	28.08.2017	-2.9	9.0	7.4	307	116	7
CG3	Harrisonburg (VA)	Center	38.42 78.86	10	29.09.2017	2.2	13.6	11.8	281	10	10
CG4	Winston-Salem (NC)	Southern edge	36.12 80.28	41	07.12.2017	5.2	15.3	14.3	321	0	11
CG5	Athens (GA)	Beyond southern edge	33.90 83.38	152	19.10.2017	6.5	16.6	16.6	375	0	12

The list reports: the abbreviation of the transplant site (CG, common garden); the location; the position within the distribution area of *A. lyrata*; the coordinates; distance to the closest known natural population; the date of sowing; minimum temperature in early spring (T_{min}espr); mean annual temperature measured with loggers at each site, and extracted from WorldClim (†); precipitation of the wettest quarter (PrecWQ); the number of days with snow cover; and the growing season as the number of months when average monthly temperature was higher than 5°C.

Table S4. Summary of the total number of cross families and seeds sown per population in each common garden (CG)

Population	N° of cross families	Seeds sown in each transplant site				
		CG1	CG2	CG3	CG4	CG5
IA1	9	70	72	57	72	66
IN1	11	72	72	72	72	72
MD2	10	69	69	69	72	69
MO1	12	72	72	69	72	72
MO2	12	72	72	72	72	72
NC2	12	69	70	72	72	72
NC4	11	72	72	72	72	72
NY1	9	70	69	63	69	66
NY4	10	70	69	69	72	72
NY5	12	72	72	72	72	72
NY5	12	72	72	72	72	72
NY6	12	72	72	66	72	63
ON1	12	72	72	72	72	69
ON11	12	72	69	66	72	66
ON12	12	69	69	72	72	72
ON3	8	69	69	69	72	66
ON8	6	27	30	27	37	27
PA3	11	72	72	72	72	72
VA1	9	72	72	72	72	72
WI1	10	66	72	72	72	72
WV1	12	69	69	66	72	60

Table S5. Description of the traits measured and their analyses

Trait	Category	Level	Explanation	Measured			Analysis
Multiplicative performance				From	To		
MP $_{\text{to year 2}}$	Continuous	Pot	Germination rate * repro. output year 2	Sowing	Summer year 2		MCMC
MP $_{\text{to year 3}}$	Continuous	Pot	Germination rate * (repro. output year 2 + 3)	Sowing	Summer year 3	†	MCMC
Demographic rate							
λ	Continuous	Pop	Finite rate of increase				REML
r	Continuous	Pop	Growth rate, log-e transformation of λ				REML
Germination & survival							
Germination	Binary	Seed	Plants germinated (0/1)	Sowing day (day 0)	Spring year 2	‡	REML
Survival $_{\text{year1}}$	Binary	Seed	Survival until end winter year 1 (0/1)	Germination	Snowmelt or soil T°>5		REML
Survival $_{\text{year 2}}$	Binary	Seed	Survival spring year 2 to spring year 3 (0/1)	End of winter year 1	Snowmelt or soil T°>5	†	REML
Reproduction							
Time to flowering $_{\text{year 2}}$	Continuous	Pot	Number of days to flower	Snowmelt or soil T°>5	Spring/summer year 2		REML
Reproductive output $_{\text{year 2}}$	Continuous	Pot	Sum of flowers and buds in year 2	Once, 9 weeks after flowering			REML
Reproductive output $_{\text{to year 3}}$	Continuous	Pot	Sum of flowers and buds in year 2 + year 3	Spring/summer year 2	Spring/summer year 3	†	REML
Root length $_{\text{year 3}}$	Continuous	Pot	Length of the longest root, in mm	Once, 5 weeks after flowering		†	REML
Damage in year 2							
Damage to rosettes	Binary	Pot	Damaged rosettes (0/1)	Once, 9 weeks after flowering			REML
Damage to inflorescences	Binary	Pot	Damaged inflorescences (0/1)	Once, 9 weeks after flowering			REML
Damage severity $_{\text{rosettes}}$	Categorical	Pot	Severity of damage categorized from 1 to 4	Once, 9 weeks after flowering			REML
Damage severity $_{\text{inflorescences}}$	Categorical	Pot	Severity of damage categorized from 1 to 4	Once, 9 weeks after flowering			REML

† except for CG3: no data after summer 2018
‡ except for CG1: second cohort in spring 2018

Table S6. Summary of means with standard error (SE) of traits measured in each of the five common gardens (CG)

	CG1 (Beyond north)		CG2 (North, edge)		CG3 (Centre)		CG4 (South, edge)		CG5 (Beyond south)	
Trait	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Multiplicative performance										
MP $_{\text{to year 2}}$	52	8	22	3	69	12	54	23	13	3
MP $_{\text{to year 3}}$	44	8	20	3	69	12	8	2	7	2
Demographic rate										
λ	1.89	0.14	1.15	0.09	1.70	0.17	1.20	0.17	0.98	0.15
r	0.57	0.09	0.07	0.10	0.42	0.12	-0.14	0.23	-0.53	0.32
Germination & survival										
Germination	0.33	0.03	0.65	0.04	0.71	0.03	0.82	0.03	0.75	0.03
Survival $_{\text{year1}}$	0.75	0.02	0.61	0.02	0.37	0.02	0.70	0.01	0.27	0.03
Survival $_{\text{year 2}}$	0.34	0.03	0.06	0.01	0.24	0.03	0.04	0.01	0.005	0.002
Reproduction										
Time to flowering $_{\text{year 2}}$	26	1	27	1	25	1	54	2	56	4
Reproductive output $_{\text{year 2}}$	137	16	33	4	154	22	35	7	95	30
Reproductive output $_{\text{to year 3}}$	172	18	35	4	154	22	198	65	175	47
Root length $_{\text{year 3}}$	131	5	59	6	NA		193	18	285	52
Damage in year 2										
Damage to rosettes	0.51	0.05	0.82	0.04	0.35	0.04	0.32	0.06	0.33	0.08
Damage to inflorescences	0.59	0.05	0.79	0.04	0.94	0.06	0.38	0.05	0.44	0.05
Damage severity $_{\text{rosettes}}$	0.62	0.04	0.20	0.04	0.74	0.03	0.38	0.09	0.65	0.09
Damage severity $_{\text{inflorescences}}$	0.27	0.02	0.44	0.07	0.26	0.01	0.27	0.04	0.29	0.04

Means were calculated based on population means of family means.

Table S7. Summary of models testing for the effect of common garden on population growth rate and several plant traits of different life stages in a transplant experiment of *Arabidopsis lyrata*

Dependent variable	*N*	CG1 (Beyond north) Estimate		CG1 (Beyond north) SE	CG2 (North, edge) Estimate		CG2 (North, edge) SE	CG4 (South, edge) Estimate		CG4 (South, edge) SE	CG5 (Beyond south) Estimate		CG5 (Beyond south) SE
Demographic rate													
r	100	0.153		0.219	-0.35		0.219	**-0.562**	*	0.219	**-0.952**	***	0.235
Germination & survival													
Germination	7,098	**-1.722**	***	0.239	**-0.513**	*	0.237	**0.795**	***	0.241	0.167		0.238
Survival $_{\text{year 1}}$	3,844	**2.424**	***	0.480	**1.602**	***	0.463	**1.736**	***	0.460	-0.626		0.458
Survival $_{\text{year 2}}$	2,205	**-1.062**	*	0.521	**-3.355**	***	0.546	**-3.614**	***	0.532	**-5.045**	***	0.685
Reproduction													
Time to flowering $_{\text{year 2}}$	1,073	0.276		1.633	1.021		1.601	**24.805**	***	1.886	**32.620**	***	2.317
Reproductive output $_{\text{year 2}}$	1,256	-19.747		20.019	**-137.534**	***	19.398	**-135.530**	***	21.955	**-88.346**	***	26.539
Reproductive output $_{\text{to year 3}}$	1,256	5.713		39.087	**-133.515**	***	38.168	62.822		42.167	6.139		49.748
Root length $_{\text{year 3}}$	226				**-66.875**	***	12.666	**82.772**	***	11.658	**150.498**	***	28.645
Damage in year 2													
Damage to rosettes	1,255	**0.305**	***	0.104	**0.585**	***	0.104	0.073		0.107	0.007		0.114
Damage to inflorescences	1,079	0.013		0.154	**-0.386**	*	0.153	-0.184		0.158	0.027		0.166
Damage severity $_{\text{rosettes}}$	676	**-0.303**	***	0.059	**-0.118**	*	0.056	**-0.540**	***	0.071	**-0.477**	***	0.091
Damage severity $_{\text{inflorescences}}$	527	0.021		0.069	**0.185**	***	0.072	0.010		0.075	0.049		0.078

The effect of each common garden was compared with the one in the centre of the distribution (CG3; except for root length – CG1). Germination, survival, damage to rosettes or inflorescences were binary variables, all other variables were continuous. Test statistics include regression coefficients of each fixed effect (*estimate*) and standard error (*SE*). Coefficients are written in bold when $P < 0.05$. Significance is indicated: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. The *bobyqa* optimizer was used to improve model converge.

Table S8. Summary of models testing for the effect of the absolute difference in minimum temperature in early spring between site of origin of populations and transplant site, $|\Delta_{Temp}|$, the absolute difference in precipitation of the wettest quarter, $|\Delta_{Prec}|$, genomic diversity depicted by Tajima's π and interactions on several plant traits in a transplant experiment of *Arabidopsis lyrata*

		$\|\Delta_{Temp}\|$		$\|\Delta_{Prec}\|$		Tajima's π		$\|\Delta_{Temp}\|*\pi$		$\|\Delta_{Prec}\|*\pi$	
Dependent variable	*N*	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Germination & survival											
Germination	7,098	**-0.063** ***	0.019	0.001	0.003	-0.057	0.091	-2E-04	0.005	-1E-05	0.001
Survival year 1	3,844	-0.041	0.030	-0.004	0.003	0.078	0.068	-0.007	0.009	-5E-04	0.001
Survival year 2	2,205	-0.037	0.048	**-0.019** *	0.009	-0.023	0.130	-0.004	0.014	0.002	0.003
Reproduction											
Time to flowering year 2	1,073	0.476	0.293	0.079	0.049	-0.704	0.683	0.017	0.080	-0.007	0.013
Reproductive output year 2	1,256	2.884	3.928	0.155	0.617	16.988 (*)	9.084	-1.332	1.107	0.058	0.164
Reproductive output to year 3	1,256	-11.789 (*)	6.830	-1.380	1.068	1.903	15.072	1.104	1.926	0.267	0.285
Root length year 3	226	-5.610 (*)	3.321	-0.047	0.759	-4.948	8.921	1.067	0.925	0.196	0.198
Damage in year 2											
Damage to rosettes	1,255	0.004	0.010	-0.001	0.002	0.003	0.022	-0.003	0.003	2E-05	-4E-04
Damage to inflorescences	1,079	0.011	0.011	**-0.004** *	0.002	0.022	0.022	-0.005	0.003	0.001	0.001
Damage severity rosettes	676	0.017	0.011	0.002	0.002	0.027	0.028	-0.005 (*)	0.003	-0.001	0.001
Damage severity inflorescences	527	-0.005	0.007	0.001	0.001	-0.012	0.014	0.003 (*)	0.002	-4E-04	-3E-04

Germination, survival, damage to rosettes or inflorescences were binary variables, all other variables were continuous. Test statistics include regression coefficients of each fixed effect (*estimate*) and standard error values (*SE*). Coefficients are written in bold when $P< 0.05$. Significance is indicated: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. The *bobyqa* optimizer was used to help models to converge.

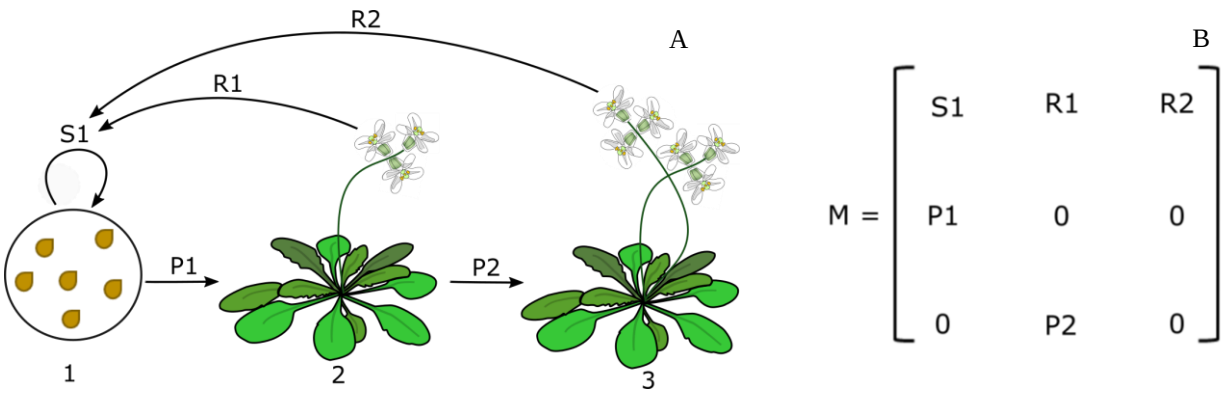

Figure S1: Estimation of population growth. For each population and common garden site, a stage-classified matrix (**panel B**) was constructed assuming three plant stages (**panel A**). The three stages were: 1– healthy seeds, 2– plants in spring of year 2 (2018), 3– plants in spring of year 3 (2019), with a projection interval for each stage set to one year. Survival between stage 1 and 2 (*P1*) was estimated as: germination rate x survival rate from the seedling stage to the reproduction period in year 2. Survival between stage 2 and 3 (*P2*) was calculated as: the survival rate from the first reproductive season to the second reproductive season (year 3). Seeds that did not germinate in the first year could have survived over winter and contributed to the seed pool of the next year, defined as the probability to remain at the same stage (*S1*). This was calculated based on a **seed-burial experiment*** over one winter. The probability to remain at the same stage was set to 0 for both stage 2 and 3, assuming that no plants survived after the third year. Reproduction in stage 2 and 3 (*R1* and *R2* respectively) were estimated as: probability to reproduce x number of fruits (plus fruits that were expected from flowers and buds) x number of healthy seeds per fruit that end in an environment suitable for germination. As we did not have any information on the latter term, we assigned to all populations a standard value leading to an average finite rate of increase in one time-step, λ, of 1 across common gardens. Estimates were $\log_e$-transformed, revealing the population growth rate, *r*.

***Seed-burial experiment**

One hundred healthy seeds per population coming from five to twelve different family lines of a population were pooled and then packed in 10 bags (nonwoven polypropylene-felt, 40 g/m^2), with 10 seeds each. Bags were brought to the transplant sites in fall 2018. In each of the five common gardens, the two bags of each population were split between two spatial blocks. Bags were placed on the ground and covered with a thin mixture of sand and peat. Seeds experienced the same ecological conditions as the plants in the transplant site until early summer 2019. Then bags were collected and examined. We distinguished between germinated and non-germinated seeds. Those seeds that had not germinated were stratified on paper disks saturated with 1.5 ml of 0.05% gibberellic acid in Petri-dishes for 10 days at 4 °C, and no light. Then, germination was assessed once every two days over a period of 20 days. *Seed survival over winter* was calculated for each replicate bag as: (germinated seedlings + germinated seedlings with gibberellic acid)/10.

Chapter 2: **Expressed mutational load increases toward the edge of a species' geographic range**

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Abstract

There is no general explanation for why species have restricted geographic distributions. One hypothesis posits that range expansion or increasing scarcity of suitable habitat result in accumulation of mutational load due to enhanced genetic drift, which constrains population performance towards range limits and further expansion. We tested this hypothesis in the North American plant, *Arabidopsis lyrata*. We experimentally assessed mutational load by crossing plants of 20 populations from across the entire species range and by raising the offspring of within- and between-population crosses at five common garden sites within and beyond the range. Offspring performance was tracked over three growing seasons. The heterosis effect, depicting expressed mutational load, was increased in populations with heightened genomic estimates of load, longer expansion distance or long-term isolation, and a selfing mating system. The decline in performance of within-population crosses amounted to 80%. Mutation accumulation due to past range expansion and long-term isolation of populations in the area of range margins is therefore a strong determinant of population-mean performance, and the magnitude of effect may be sufficient to cause range limits.

Introduction

What determines the limits of species' geographic distributions has been a long-standing question in biology, yet the more ultimate evolutionary causes are still not fully understood (Gaston 2009; Sutherland et al. 2013; Connallon and Sgrò 2018; Willi and Van Buskirk 2019). Ecological research has focused on limiting environmental factors and used the concept of the ecological niche of species to understand range limits (e.g., Hargreaves et al. 2014; Lee-Yaw et al. 2016). In contrast, evolutionary theory has focused on constrains in adapting to ecological gradients, for which few direct empirical tests exist to date (recent theory: e.g., Polechová and Barton 2015; Polechová 2018; older theory and empirical work reviewed in: Kawecki 2008; Gaston 2009; Sexton et al. 2009). Another evolutionary explanation for distribution limits is enhanced genetic drift and the accumulation of deleterious mutations towards the range edge, due to a history of small population size either produced by past range expansion or a scarcity of suitable habitat (Peischl et al. 2013; Peischl and Excoffier 2015; Henry et al. 2015; reviewed in Willi 2019). These theoretical studies have described conditions favoring mutational load in contributing to range limits, but few empirical estimates of mutational load across species distributions have been made and the fitness consequences of mutational load in nature are unknown. If the phenotypic effect of mutational load due to past expansion or habitat scarcity is considerable, it may constrain population persistence and establish a range limit by preventing further expansion (Peischl et al. 2015; Henry et al. 2015).

Populations at range edges may often have a history of small size, with the predicted consequence of heightened genetic drift that erodes genetic variation and opposes the effect of (mostly weak) selection (Wright 1931; Kimura et al. 1963). In long-term small populations, the consequence of drift opposing purifying selection is the accumulation of deleterious mutations, leading to a reduction in fitness called mutation(al) load (Kimura et al. 1963). Similar to stable small population size, demographic bottlenecks are also expected to enhance genetic drift, erode

genetic variation (Nei et al. 1975), and heighten mutational load (Kirkpatrick and Jarne 2000). Recent theoretical work by Peischl and co-workers suggested that serial bottlenecks during rapid range expansion lead to the accumulation of mutational load, in this context termed expansion load, which decreases population mean performance and slows down expansion or even halts expansion if recombination is low (Peischl et al. 2013, 2015; Peischl and Excoffier 2015). The increased frequency of recessive deleterious mutations contributes strongest to mutational load (Peischl and Excoffier 2015) and load can persist for thousands of generations (Peischl et al. 2013). Most notably, predictions of this general model apply in the absence of any environmental gradient. A different type of neutral model also predicted stable range margins due to mutation accumulation along a gradient of habitat quality. Henry et al. (2015) performed simulations along linear arrays of habitat patches of decreasing carrying capacity and found that the range limits retract to a stable point, before reaching the limit of habitat patches, due to mutation accumulation if both dispersal and population growth rate are small.

Empirical research suggests that a history of past range expansion is common in many taxa and that the habitat often deteriorates at range edges, both of which are associated with enhanced genetic drift. Quaternary ice ages caused retraction of the geographic distribution of many species into refugia, from which they have re-expanded, leaving many with distribution margins characterized by a history of recent range expansion and lowered effective population size (Hewitt 2000). Furthermore, several recent meta-studies confirmed the general trend for enhanced habitat deterioration and habitat isolation towards and around the geographic range limits and lower effective population sizes. A meta-study on transplant experiments with sites beyond the range edge revealed significant performance declines beyond range edges in about 80% of studies (Hargreaves et al. 2014), which was paralleled by a decline in habitat suitability deduced by niche modelling (Lee-Yaw et al. 2016). Furthermore, the density of individuals and populations of species were found to generally decline towards the range edge (Pironon et al.

2017). Other meta-level studies show that populations at range edges have reduced within-population genetic marker variation and are genetically more differentiated, documenting the enhanced action of genetic drift (Eckert et al. 2008; Sexton et al. 2009; Pironon et al. 2017). In the context of range margins, the evolution of mating system shifts received additional attention. In hermaphroditic organisms, the incidence of self-fertilization increases towards range edges due to a history of mate limitation and the lowering of inbreeding load (Pujol et al. 2009; Griffin and Willi 2014; Matos et al. 2015). One consequence of a shift to selfing is increased genetic drift (Pollak 1987; Nordborg and Donelli 1997) and mutation accumulation (Lynch et al. 1995a; Schultz and Lynch 1997). Indeed, estimates of effective population sizes are typically lower in selfing compared to outcrossing taxa (Ingvarsson 2007; Hartfield et al. 2017).

The accumulation of deleterious mutations during range expansion has been studied best in humans. Populations with a longer history of expansion out-of-Africa, European Americans, had higher proportions of non-synonymous to all single-nucleotide polymorphisms (SNPs) compared to African Americans (Lohmueller et al. 2008). Similarly, an increased frequency of predicted deleterious mutations was observed in out-of-Africa populations compared to humans from southern Africa (Henn et al. 2016). In plants, increased genomic estimates of mutational load with range expansion have been described in at least three species (González-Martínez et al. 2017; Willi et al. 2018; Koski et al. 2019). However, empirical evidence of the link between expansion history and performance decline are scarce. In an experimental-evolution study with bacteria, lines with high mutation rates evolved to have reduced growth under range expansion over 1650 generations compared to their ancestral lines, suggesting accumulation of mutational load (Bosshard et al. 2017). Increased genomic estimates of mutational load towards the distribution edge were associated with reduced performance assessed in a common garden in the species *Arabidopsis lyrata* (Willi et al. 2018).

In *Campanula americana*, populations further away from a putative glacial refugium in the southern Appalachians expressed increased mutational load in the greenhouse (Koski et al. 2019). However, these studies have not tested the contribution of mutational load to reducing population performance and demographic rates under natural conditions or across the distribution of a species. Moreover, the life stage at which mutational load is expressed is not known (Hansen and Price 1999). Based on theory, we expect that the cumulative effects of numerous deleterious mutations each of small effect become most detectable at later life stages (Husband and Schemske 1996).

In this study, we estimated the expression of mutational load of natural populations of *A. lyrata* subsp. *lyrata* (L.) from across the species range in common gardens within and beyond the distribution range. The species is ideal for investigating genetic causes of range limits because niche modelling has shown that the species is not dispersal-limited in the south and north, indicating that range limits reflect niche limits (Lee-Yaw et al. 2018). Furthermore, previous population genomics studies demonstrate a history of fast post-glacial range expansion from two distinct refugia, resulting in two genetically distinct clusters with a small contact zone at Lake Erie (Willi and Määttänen 2010; Griffin and Willi 2014; Willi et al. 2018). Distance of expansion or rear-edge distance to the glacial refugia was positively associated with genomic estimates of mutational load, indicating that both past range expansion and long-term isolation at the south-western range edge left a signature of mutation accumulation. The highest genomic estimates of mutational load were found in selfing populations, which in this species are restricted to areas at or close to the edge of the range (Griffin and Willi 2014; Willi et al. 2018). To estimate expressed mutational load, we used the proxy of heterosis, *i.e*. the increase in fitness of between-population crosses compared to within-population crosses due to increased heterozygosity of recessive deleterious mutations (dominance model of heterosis, Crow 1987). We tested the following predictions: (i) Mutational load expressed in the field is tightly

correlated with mutational load estimated on a genomic level. (ii) As with genomic estimates of load, expressed mutational load is correlated with postglacial expansion distance or long-term isolation at the rear edge and with mating system. (iii) Expressed mutational load is based on weakly deleterious mutations, whose cumulative effect is greatest at late life stages.

Material and Methods

Plant material and the crossing of plants

Twenty populations of *A. lyrata* subsp. *lyrata* were selected to represent the whole range of distribution of the species (Fig. 1, Table S1). They represented: the two genetic clusters of the species in North America; different histories during and since the last glaciation cycle, either one of being close to glacial core distribution or one of expansion or rear-edge isolation; different mating systems, either being predominantly outcrossing or predominantly selfing (Griffin and Willi 2014). Seeds of different maternal plants per population were collected between 2007 and 2014 over an area of about 450 m^2 in each population. Seeds had been stored in separate bags per maternal plant at 4 °C under dry, dark conditions.

We raised 26 plants per population in growth chambers, one per field-collected maternal plant and that we assumed were unrelated, for the production of within- and between-population crosses. Three seeds per maternal plant were initially sown in individual pots filled with a 1:1 mixture of sand and peat. Pots were watered to saturation and seeds stratified for 12 days at 4 °C in the dark. Pots were then transferred to growth chambers (CLF Plant Climatics, Wertingen, Germany) with the following conditions to promote germination: 8h of light at 100 µmol m^{-2} s^{-1} and 20 °C, 16h of dark at 20 °C. Germinated plants were thinned to one per pot, 36 days after sowing. To promote growth and flowering, day length and light intensity were increased every three days by 1h and 20 µmol m^{-2} s^{-1}, respectively, over a period of 25 days, and day temperature was increased by 2 °C. The final conditions were kept until the end of the crossing experiment:

16h of light at 240 μmol m^{-2} s^{-1} and 22 °C, 8h of dark at 20 °C. After 25 days, when the first individuals started to bolt, all pots were transferred to a greenhouse with similar conditions as in the growth chambers to perform the crosses (all growth conditions detailed in Table S2).

Of the 20 populations, 18 were considered target populations, and two served as pollen donors for between-population crosses. The latter two populations were located in the center of distribution of the two ancestral clusters and had high genomic diversity (NY1 for the eastern cluster, IA1 for the western cluster). For each of the 18 target populations, 12 of the 26 individuals were randomly chosen as being "mothers" (pollen recipients) and 12 individuals as "fathers" (pollen donors); the remaining plants were used as backups. The 12 mother plants of a target population were crossed with pollen from a randomly chosen father plant of the same population (WPC) and from a randomly chosen plant of the partner population (BPC); crosses were non-reciprocal. WPC crosses were also performed for the two partner populations (list of families and cross combinations in Table S3). We made hand-pollinations at the bud stage to exclude unwanted cross- and spontaneous self-pollination. Flower buds of the mother plant were opened with tweezers, the immature anthers were removed, and mature anthers of a father plant gently rubbed over the stigma. Pollen contamination was avoided by sterilizing the tweezers after each contact with a flower, and placing each pollinated plant into an insect-proof growth chamber until fruit elongation began (3-5 days). Each cross combination was repeated to obtain a sufficient number of seeds for the outdoor common gardens (at least six siliques or 60 healthy-looking seeds). Cross combinations were changed if no siliques or no viable seeds could be obtained. We collected mature siliques and left them to dry for two weeks at ambient temperature in the dark. Afterwards, they were stored at 4 °C, under dry and dark conditions.

Raising of plants in common gardens

Expressed mutational load, the heterosis effect in F1 individuals, was assessed at five common garden sites along a 1400 km latitudinal gradient in the eastern USA (Fig. 1). One site was in

the center of the range of *A. lyrata*, in Harrisonburg, VA, two sites were close to the southern and northern borders of the range, in Winsten-Salem, NC, and Williamstown, MA, respectively, and two sites were beyond the southern and northern range edge, in Athens, GA, and the Adirondacks, NY, respectively (Table S4). In the analyses presented here, sites were treated as a level of replication for estimating mutational load. Our main goals were to analyze the relationships between expressed mutational load and a genomic estimate of mutational load (prediction i) and between expressed mutational load and past range dynamics or mating system (prediction ii). The common garden study started in fall 2017 and used the same protocol for each garden, with slight deviations due to local facilities. We sowed seeds from all successful cross combinations that had more than 15 healthy seeds in each garden. If a cross combination failed to produce enough viable seeds, we added an additional cross combination from the same population with a sufficient number of seeds. In total, 401 cross combinations contributed to the field experiment (Table S3). Per cross combination and common garden, three pots were filled each with two seeds (in some cases only one seed was available). Pots were randomly positioned across thirteen 38-cell propagation trays within each of three blocks per common garden. Across the five gardens, a total of 12,933 seeds were sown. In all common gardens, we used the same substrate mixture of washed river sand and peat (1:1.5 sand:peat). Sowing was done in early fall to early winter and started at the northernmost site. To prevent seeds from being washed away by heavy rainfall, germination was carried out under a ventilated greenhouse or temporary tent for 17-19 days until the peak of germination was reached. The trays were then exposed to natural conditions for the rest of the experiment. During fall 2017, the trays were regularly watered during periods of no rain, to ensure a constant moisture of the substrate, until snow fell or the first night frosts occurred. We weeded the pots manually, and seedlings were thinned starting 11 weeks after sowing to keep only one individual per pot. Herbivory by grazing was prevented by a fence, and organic slug repellent was used in the

beginning of spring, after snowmelt. No further interventions were made until the end of the experiment in summer 2019 (2018 for Harrisonburg because the garden was needed for another experiment).

We measured performance on the level of the individual pot/plant. Day of germination, when a seedling had two fully open cotyledons, was checked three times a week until the peak of germination was over (4-5 weeks after sowing) and then once a week until the first thinning. Germination was again checked in spring 2018. Death of seedlings was recorded at the same time as germination was checked, and later, mortality was checked once a week unless there was a snow cover. We scored the day of first flower opening three days a week, starting when bolting was observed in 2018. Day of germination, death, and flowering were corrected by the mid-time between previous checking and actual observation. Reproductive output was estimated in 2018 and 2019 by counting the number of fruits, pedicels (flowers that did not develop into a fruit), open flowers, and flower buds on all inflorescences. Female reproductive output of each individual was the total number of fruits and potential additional fruits that could have formed from buds and open flowers: fruits + ((flowers + buds) × (fruits / (fruits + pedicels))). We assessed reproductive output several weeks after peak flowering: in 2018 ~ 9 weeks after opening of the first flowers within each common garden, and in 2019 ~ 5 weeks after first flowering, estimated from flowering dates of the previous year.

To assess the contribution of the seed bank to population growth, we carried out a seed survival experiment over the winter of 2018/19. One hundred healthy seeds of five to twelve mother plants from each WPC and BPC cross combination were pooled on the level of the population and cross type, and packed in groups of 10 seeds in 10 separate bags made out of micro-perforated fabric (nonwoven polypropylene-felt, 40 g/m^2) that allowed the penetration of air and moisture. Two bags of each pool were placed in each of the five common gardens in October 2018 on freshly weeded and homogenized soil next to the pots to expose them to

natural conditions, and they were retrieved in late spring 2019. Each pool was then visually screened to discriminate between seedlings and seeds. We then judged survival by first stratifying seeds on filter paper disks soaked with 1.5 ml of 0.05% gibberellic acid in petri-dishes (10 days, 4 °C, no light). Germination was assessed under similar conditions as detailed for the crossing experiment and scored over 20 days. Seed survival over winter was then estimated for each bag as: (germinated seedlings + germinated seedlings with gibberellic acid)/10.

Statistical analysis

We analyzed two measures of performance, using pot as the level of replication. *Multiplicative performance I* was the fraction of seeds that germinated multiplied by the total reproductive output in year 2 plus in year 3, and *multiplicative performance II* was germination multiplied by the number of fruits only. Components of these overall performance estimates were analyzed separately and are described in Table S5; these analyses were used to identify the life stages most impacted by mutational load (prediction iii). Finally, to assess how mutational load affected demography, we estimated population growth rates for all WPC (20) and BPC combinations (18) in each common garden by constructing stage-classified matrices (Caswell 2001), based on population mean data of each common garden. The matrices were composed of three stages: 1–healthy seeds, 2–individuals capable of reproducing in spring of year 2 (2018), 3–individuals capable of reproducing in spring of year 3 (2019), with a projection interval set to one year for each stage. The exact parametrization of the matrices is described in Fig. S6. For each combination of population, cross type and common garden, we calculated λ, the finite rate of increase in one time-step (Caswell 2001).

Preliminary analyses on the level of the pot/plant (described below) revealed that the effect of cross-type was highly significant for *multiplicative performance I* and *II*, and therefore

we present the analyses and results on heterosis first. Population-level heterosis was calculated as the increase in performance due to between-population crossing relative to within-population crossing, as follows: $(W_{BPC} - W_{WPC})/W_{WPC}$. W_{WPC} and W_{BPC} were calculated for each population in each common garden based on family means. In the case of W_{WPC}, the final value was an average of the two types of WPC, of the target and the pollen-donor population. In case either W_{BPC} or W_{WPC} was equal to zero for a specific cross combination in a specific common garden, we chose to replace this value by the smallest non-zero value observed within cross type (12 cases for *survival summer year 2*, three cases for *survival winter year 2*). Heterosis estimates were $\log_{10}$-transformed (after making all values positive by adding +1), and tested by hierarchical mixed-effects models using restricted maximum likelihood with the packages lme4 (Bates et al. 2015) and LmerTest (Kuznetsova et al. 2017; model parametrization in Appendix S7A) in *R* (R Core Team 2019). Fixed effects were either the genomic estimate of mutational load, or the recent range-dynamics history of a population and mating system. The genomic estimate of mutational load (hereafter genomic load) was the ratio of non-synonymous polymorphic sites to synonymous polymorphic sites, adjusted for their mean derived allele frequency relative to *A. thaliana*, $P_n f_n / P_s f_s$ (Willi et al, 2018). The range-dynamics history of a population was its $\log_{10}$-transformed distance to distribution cores. Cores were glacial refugia that gave rise to range expansion, identified by means of the map-projection of a population phylogeny. More precisely, cores were defined as the location of the ancestral node from which a first ancestral population appeared that was located in an area covered by ice during the last glacial maximum (Willi et al. 2018). For younger populations, distance to core was calculated as the sum of great circle distances [km] from the location of the extant population back along the map-projected phylogeny to the core and reflected the expansion distance. Populations that had diverged earlier were considered rear-edge relative to the core sites. For these, the direct great-circle distance to the ancestral core population was calculated. The two Missouri

populations, although part of a separate third cluster, were considered as being part of the western cluster due to proximity and a closer shared history of admixture (Willi et al. 2018). As a proxy for mating system we used the population inbreeding coefficient, F_{IS} (Griffin and Willi 2014). Continuous fixed effects were mean-centered before running each analysis. The random part of models included the crossed effects of maternal population and common garden.

Further analyses validated the use of heterosis as a proxy of expressed mutational load. First, we verified consistency in results between population-level analyses and pot/plant-level analyses. Dependent variables were the two measures of *multiplicative performance* and the separate performance components. Fixed effects were cross type, genomic load, and their interaction. Preliminary analyses showed that the best random structure was: maternal plant nested within maternal population and maternal population, for which intercepts and slopes of cross type were estimated, and block nested within common garden, and common garden. The two *multiplicative performance* variables were 0 inflated, which suggested the modelling of two processes, a Gaussian process (for $\log_{10}$-transformed performance values > 0), and a logistic process (modelling the probability of 1, assigned to performance values > 0). Analyses were performed in a Bayesian framework, with the package MCMCglmm (Hadfield 2010, 2019) on 10 parallel chains (model and prior parametrization detailed in Appendix S7B). Analyses on variables depicting life stage components made use of restricted maximum likelihood (model parametrization detailed in Appendix S7C). Next, analyses were repeated on ($\log_{10}$-transformed) population means for each cross type and each common garden, by use of restricted maximum likelihood. Fixed effects were cross type, mean-centred genomic load and the interaction between the two. Crossed random effects were maternal population and common garden. To validate if heterosis is the result mainly of dominance and load due to fully recessive deleterious mutations, we tested for a relationship between W_{WPC} and genomic load, and W_{BPC} and genomic load similar to above.

Results

Overall, 64.2% of all seeds germinated (Table S8). Plants had high survival rates at each life stage (61.8-99.6%), except for *survival summer year 2*, which was the most critical life stage (29.8%), with most deaths happening after reproducing. Surprisingly, despite high *survival to flowering year 2* (99.6%), only 60.2% initiated flowering, while 95% of plants that survived to year 3 initiated flowering (data not shown). Finally, individuals that flowered in year 2 produced on average 135 flowers, with values ranging from 1 to 2607 flowers, with an average fertilization rate of 67.2%. Heterosis in *multiplicative performance I* and *II* up to year 3, assessed per population and common garden, ranged from -0.96 to 23.50 (mean: 1.88) and from -0.92 to 30.23 (mean: 2.65), respectively (Table S8). Finally, heterosis estimated on λ was between -0.53 and 7.29 (mean: 0.73; Table S8).

Expressed mutational load, here estimated by heterosis in *multiplicative performance I* and *II* up to year 3, was positively related with the genomic estimate of mutational load (Table 1, Fig. 2; results on *MP I* and *II* to year 2 reported for comparison). The model-predicted increase between the population with the lowest and that with the highest genomic load was up to 5.6-fold (Table S9). Also, heterosis in *multiplicative performance I* and *II* up to year 3 significantly increased with the distance between the site of origin of a population and the glacial core distribution (Table 1, Figs. 2, 3). The predicted maximal increase in heterosis between the closest and farthest population from the glacial cores was up to 3.4-fold (Table S9). Analyses on outcrossing populations confirmed the positive effect of distance to core on heterosis (Table S10). Heterosis was higher in selfing populations for *multiplicative performance I* and *II* up to year 2 but not to year 3 (Table 1). The predicted maximal increase in heterosis between the most outcrossed and the most inbred population was 3.3-fold for *multiplicative performance II* to year 2 (Table S9). The intercept of the linear models was

significant for heterosis in *multiplicative performance I* and *II* to year 3, indicating that the average population suffered from mutational load (Table 1).

Heterosis associated with genomic load was significant relatively early in life (Table 1). *Survival fall year 1* was the second variable after germination in the life stage analyses and for this variable a significant positive relationship between heterosis and genomic load was found. Further variables with a significant positive relationship between heterosis and genomic load were: *bolting*, *reproductive output* and *number of fruits* produced, all in year 2. *Germination* was the first life stage for which the relationship between heterosis and distance to core was significant (Table 1). Further variables with a significant positive relationship between heterosis and distance to core were *reproductive output* and *number of fruits* produced in year 2. Results were similar when analysis was restricted to outcrossing populations (Table S10). Heterosis in *survival fall year 1, survival winter year 2,* and *bolting* were significantly positively related with F_{IS} (Table 1). Finally, heterosis for λ was positive and significant for genomic load, and as a trend for distance to core, and for F_{IS} (Table 1, Fig. 2). The model-predicted increase between the population with the lowest genomic load to the population with the highest genomic load was 1.3-fold (Table S9). For F_{IS}, the model-predicted increase between the most outbred to the most inbred population was 1.7-fold (Table S9).

Analyses similar to those presented above were performed on the level of individual pots/plants, with cross-type in the fixed effects part of the model (Table S11). Hierarchical mixed-effects model analyses revealed a significant effect of cross type on *multiplicative performance I* and *II* to year 3 in both the log-normal process and the logistic process (Table S11A; results on *MP I* and *II* to year 2 reported for comparison). Between-population crosses (BPC) had higher performance than within-population crosses, supporting a general heterosis effect. No direct effect of genomic load on *multiplicative performances* was observed. However, the cross type-by-genomic load interaction was significantly positive in the log-

normal aspect of both *multiplicative performance* estimates; the performance of BPC declined less with genomic load than the performance of WPC. Similarly, when averaging both *multiplicative performance* estimates on the level of population for each cross type and common garden (Table S12), BPC performed significantly better than WPC. Furthermore, both *multiplicative performance* estimates were negatively related with genomic load, while again the cross type (BPC)-by-genomic load interaction had a significant positive effect on *multiplicative performance I* (marginally significant for *multiplicative performance II*). These results indicated that the relationship between performance and genomic load was more negative for WPC than BPC. Also analyzing both cross types separately confirmed the negative relationship between *multiplicative performance I* and *II* of WPC and genomic load, while no significant relationship was found for BPC (Table S13, Fig. 4). The predicted decline of WPC performance had a maximum value of 80.3% for *multiplicative performance I* to year 3(Table S9).

Discussion

Recent evolutionary theory proposes that the neutral process of genetic drift can contribute to slowing further range expansion in a species or cause stable range edges due to the accumulation of mutational load (reviewed in Willi 2019). Here we showed experimentally that both leading and rear edge populations suffered from the increased expression of mutational load – estimated by heterosis based on life-time performance, demographic rates, and performance at individual life stages. The expression of mutational load was also higher in selfing populations predominantly located at the distribution edge, aggravating the negative effect of load at range edges. Overall, this study provides empirical support for an important role of mutational load in range limits.

The expression of mutational load increased by a factor of 3.4 with distance along the expansion route or distance from the historic core of distribution towards the distribution edges of *A. lyrata*. The decline in population mean multiplicative performance of within-population crosses due to increasing genomic load was up to 80%. These results constitute some of the first *in-situ* evidence on the expression of mutational load towards range limits, and support predictions from simulation studies (Peischl et al. 2013; Peischl and Excoffier 2015), genomic data (Willi et al. 2018), or similar phenotypic data from the greenhouse or garden (Willi et al. 2018; Koski et al. 2019). The strong link between expressed mutational load, mutational load estimated with sequence data, and range position observed in our study system sheds light on the processes shaping range limits (reviewed in Willi 2019). Further colonization by leading edge populations, already suffering from high levels of load, may be impeded by additional accumulation of mutational load, reducing performance below critical thresholds necessary to maintain persisting populations. Similarly, at the rear edge, population isolation and low effective population sizes may lead to mutational melt-down (Lynch et al. 1995b), such that rear-edges are unstable over the long term and in a state of gradual retraction.

We found that the most inbred populations, the three predominantly selfing populations located at the northern, eastern, and southern edges of the western cluster, expressed even higher levels of load than outcrossing populations, with a predicted 3.3-fold maximal increase in heterosis based on multiplicative performance (up to the second year), and 1.3-fold increase in heterosis based on demographic rates. A similar result was found earlier on a different set of selfing populations of *A. lyrata* (Willi 2013). Higher levels of mutational load in selfing populations is expected due to their generally lower effective population size combined with increased exposure to genetic drift (Lynch et al. 1995a). Indeed, genomic signatures of mutational load are increased in several other selfing taxa (reviewed in Wright et al. 2013; Laenen et al. 2018). Theoretical and empirical studies predict higher rates of selfing towards

range limits (reviewed in Pannell 2015), as observed in *A. lyrata* (Griffin and Willi 2014). This overrepresentation of selfing populations at range edges could lead to a biased estimation of the effect of expansion on mutational load, but our conclusions are not affected by this because the statistical models accounted for mating system. This was also confirmed by analysis of outcrossing populations only, which produced similar effect sizes for distance to core on heterosis. The most important insight, however, is that selfing populations may often bear a double load, one from the long expansion history and one from selfing. Both are likely to increase extinction risk (Goldberg et al. 2010) and be effective in causing range limits (Peischl et al. 2015).

As predicted, our results generally supported the expectation that the correlation between load and either a genomic estimate of load or distance to core strengthened over the life cycle of the plants. In an early phase of the life cycle, survival shortly after germination showed heightened heterosis with genomic load, and germination showed heightened heterosis with distance to core. But effect sizes were weaker than those found for later life stages (Table 1). Heterosis linked to genomic load or distance to core was found consistently for several performance variables of the first reproductive period (bolting, reproductive output, and number of fruits). Finally, the strongest associations between heterosis and either genomic load or distance to core occurred in the multiplicative performance estimates. These results agree with the prediction that expression of load is due to deleterious mutations with cumulative effect over an organism's life (Husband and Schemske 1996). More and more genes contribute to performance over the course of the life of an organism, so the number of genes potentially experiencing load also increases, and this should produce a cumulative effect. Other empirical support for this model comes from studies assessing inbreeding depression in long lived perennials (e.g., Koelewijn et al. 1999; Griffin et al. 2019). Another prediction, according to theory, is that the magnitude of genetic drift determines the effect sizes of mutations that

become targets of neutral evolution and are freed from purifying selection (Kimura et al. 1963). Here our results suggest that drift associated with past range dynamics must have been very strong, allowing mutations with significant phenotypic effect to accumulate in the presumably fewer genes relevant early in life (e.g., already at the time of germination).

For selfing populations, the pattern of expression of mutational load through the life cycle is probably similar to that in outcrossing populations. Survival shortly after germination showed heightened heterosis in populations with a selfing mating system. Later life stages with significantly increased heterosis were bolting during the first reproductive season and the survival during the second winter. In a previous study including five other selfing or mixed-mating populations of *A. lyrata*, Willi (2013) reported heightened heterosis associated with selfing only in reproductive output in the third year, but not in earlier life stages. However, another study focusing only on early life stages reported lower performance of within-population crosses for germination in selfing *A. lyrata* (Joschinski et al. 2015). Overall, it seems that also in selfing populations, the magnitude of the expression of load increases over the lifetime of a plant, and that early life phases can already be affected.

Our results suggest that heterosis accurately reflects the fitness effect of mutational load. Just as the phenotypic comparison between in- and outbred individuals can accurately estimate inbreeding depression (Keller and Waller 2002), heterosis can indicate the expression of mutational load *in vivo* and, with an appropriate rearing design, *in situ.* One advantage of this approach is that other confounding effects can be excluded. For example, by using between-population crosses as the reference for performance, we control for the potential influence of population-specific local adaptation of within-population crosses. However, this method depends on two important assumptions: that heterosis is affected only by dominance (and not overdominance) and that load is primarily due to fully recessive deleterious mutations (Oakley et al. 2015; Peischl and Excoffier 2015). We verified both assumptions. The fact that

performance of between-population crosses did not increase with genomic estimates of load indicates that overdominance was not important. Likewise, the fact that performance of BPC did not decline with genomic load suggests that partially recessive deleterious mutations do not contribute appreciably to mutational load. A previous common garden study with *A. lyrata* found that part of the load was caused by partially recessive mutations (Willi et al. 2018). A further challenge is that heterosis can be affected by a performance decay due to outcrossing with distantly-related individuals, due to hybrid breakdown or disruption of coadapted gene complexes. In fact, the negative estimates of heterosis in our study, which occurred in nearly a fourth of the BPC, may reflect genetic incompatibilities such as the Dobzhansky-Muller type (Lynch 1991; reviewed in Oakley et al. 2015). Peripheral populations were generally genetically more isolated, so we assume that outbreeding depression was stronger for these populations. If this is true, our estimates of mutational load for range-edge populations would be slight underestimates, and the increasing load with post-glacial expansion distance would be even greater than reported here.

Our findings clearly show that populations with the longest expansion history suffer most from the expression of mutational load. Populations with the highest genomic signatures of load, located at both leading and rear-edges of the distribution, suffer from the expression of load to the extent that it impairs their demographic rates. The accumulation of mutational load is therefore likely to be involved in shaping range limits by impeding further expansion at the leading edge and causing retraction at the rear edge. The discovery that population history impacts population persistence at range edges argues for the integration of evolutionary history into biodiversity conservation management (Hoffmann et al. 2015). These processes are also important in the context of climate change: strong mutational load at range edges could impair expansion into newly available habitats while rear-edge populations would suffer from increasing isolation due to habitat fragmentation, mutation accumulation, and eventual

extinction. Genetic drift at range margins is predicted to limit adaptation and expansion into empty habitat (Polechová and Barton 2015; Polechová 2018). Our results imply that models of range limits along environmental gradients should integrate increasing drift and mutation accumulation towards range edges. This will produce deeper insights in the relative importance of factors contributing to maladaptation, range limits, and responses to climate change.

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Tables

Table 1: Summary of models testing for the effect of genomic estimates of mutational load or geographic distance to core and mating system (F_{IS}) on population-level heterosis at five common garden sites

		Genomic load						Distance to core [km]			F_{IS}					
Dependent variable	***N***	***Estimate***	***χ²***		***R²m***	***R²c***		***Estimate***	***χ²***		***Estimate***	***χ²***		***R²m***	***R²c***	
Multiplicative performance (MP)																
MP I to year 3	89	**1.90**	**6.99**	**	0.117	0.342	†,‡,§	**0.75**	**6.53**	*	0.32	2.05		0.175	0.350	†,‡,§
MP II to year 3	89	**2.11**	**9.30**	**	0.138	0.431	†,‡	**0.75**	**6.94**	**	0.38	3.12	(*)	0.188	0.438	†,‡
MP I to year 2	89	**2.47**	**14.31**	***	0.179	0.405	‡,§	**0.68**	**5.85**	*	**0.50**	**5.35**	*	0.200	0.414	‡,§
MP II to year 2	89	**2.52**	**14.65**	***	0.168	0.423		**0.72**	**6.52**	*	**0.51**	**5.66**	*	0.192	0.431	
Life stage components																
Germination	89	-0.10	0.19		0.005	0.465	§	**0.19**	**4.61**	*	-0.10	2.18		0.105	0.470	
Survival fall year 1	89	**0.24**	**7.38**	**	0.056	0.334		0.00	0.01		**0.10**	**10.43**	**	0.087	0.361	§
Survival winter year 1	89	0.02	0.18		0.002	0.077	‡	0.03	1.19		0.00	0.04		0.013	0.088	‡
Survival summer year 2	89	0.07	0.04		0.000	0.160		0.13	0.69		-0.03	0.06		0.007	0.161	
Survival winter year 2	44	0.27	0.45		0.010	0.010	†	-0.25	2.19		**0.29**	**4.78**	*****	0.113	0.113	†
Reproduction year 2																
Survival to flowering year 2	89	0.06	2.81	(*)	0.026	0.178	§	-0.01	1.02		0.02	3.57	(*)	0.035	0.186	
Bolting	89	**0.64**	**9.90**	**	0.067	0.408		0.05	0.3		**0.16**	**4.83**	*	0.050	0.399	
Reproductive output	82	**1.24**	**7.51**	**	0.127	0.281	‡	**0.45**	**5.4**	*	0.16	1.07		0.151	0.301	‡,§
Number of fruits	82	**1.09**	**6.26**	*	0.104	0.244	‡	**0.45**	**6.39**	*	0.13	0.87		0.150	0.263	‡
Demographic rate																
λ	89	**0.31**	**6.16**	*	0.108	0.470		0.17	2.71	(*)	**0.23**	**4.51**	*	0.160	0.476	

Population heterosis estimates (log_{10}-transformed) were assumed to follow Gaussian distributions. The effect of distance to core and F_{IS} were assessed in the same model. Test statistics include regression coefficient (*estimate*), χ^2-value and the marginal and conditional R^2 of the model. Genomic load, distance to core and F_{IS} were standardized prior to analyses (mean = 0). Estimates with *P*-values < 0.05 are written in bold; significance is indicated: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Results for random effects are not shown.

†For one of the five common gardens (CG3), the experiment stopped early and variables consider performance to year 2 only.

‡Model fits with significant (positive) intercept.

§The *bobyqa* optimizer was used when models initially failed to converge.

Figures

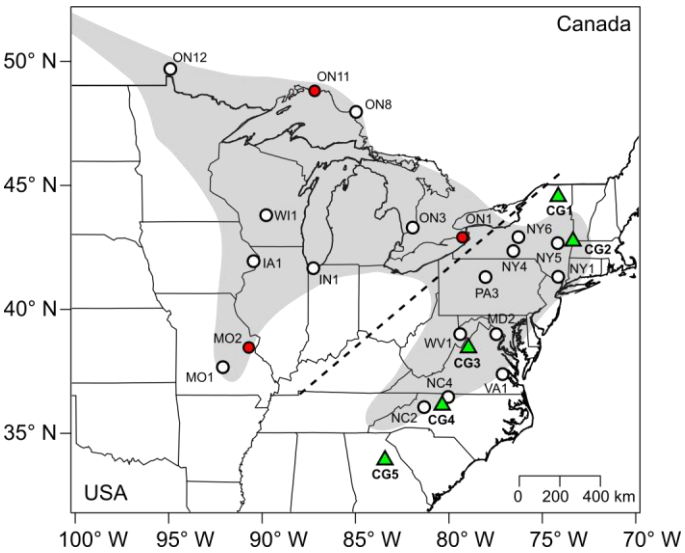

Figure 1: Distribution map of *Arabidopsis lyrata* in eastern North America with the locations of the 20 populations studied and the 5 common garden sites. Circles filled in white or red represent outcrossing and selfing populations, respectively. Population labels consist of the abbreviation for state (USA) or province (Canada) and a number (as in Willi *et al*. 2018). Green triangles represent the five common garden (CG) sites; numbers added to labels are in sequence of north to south. The dashed line is the split between eastern and western genetic clusters. Of the 20 populations, two were used as partner-populations for between-population crosses, NY1 for crosses with eastern populations, and IA1 for crosses with western populations.

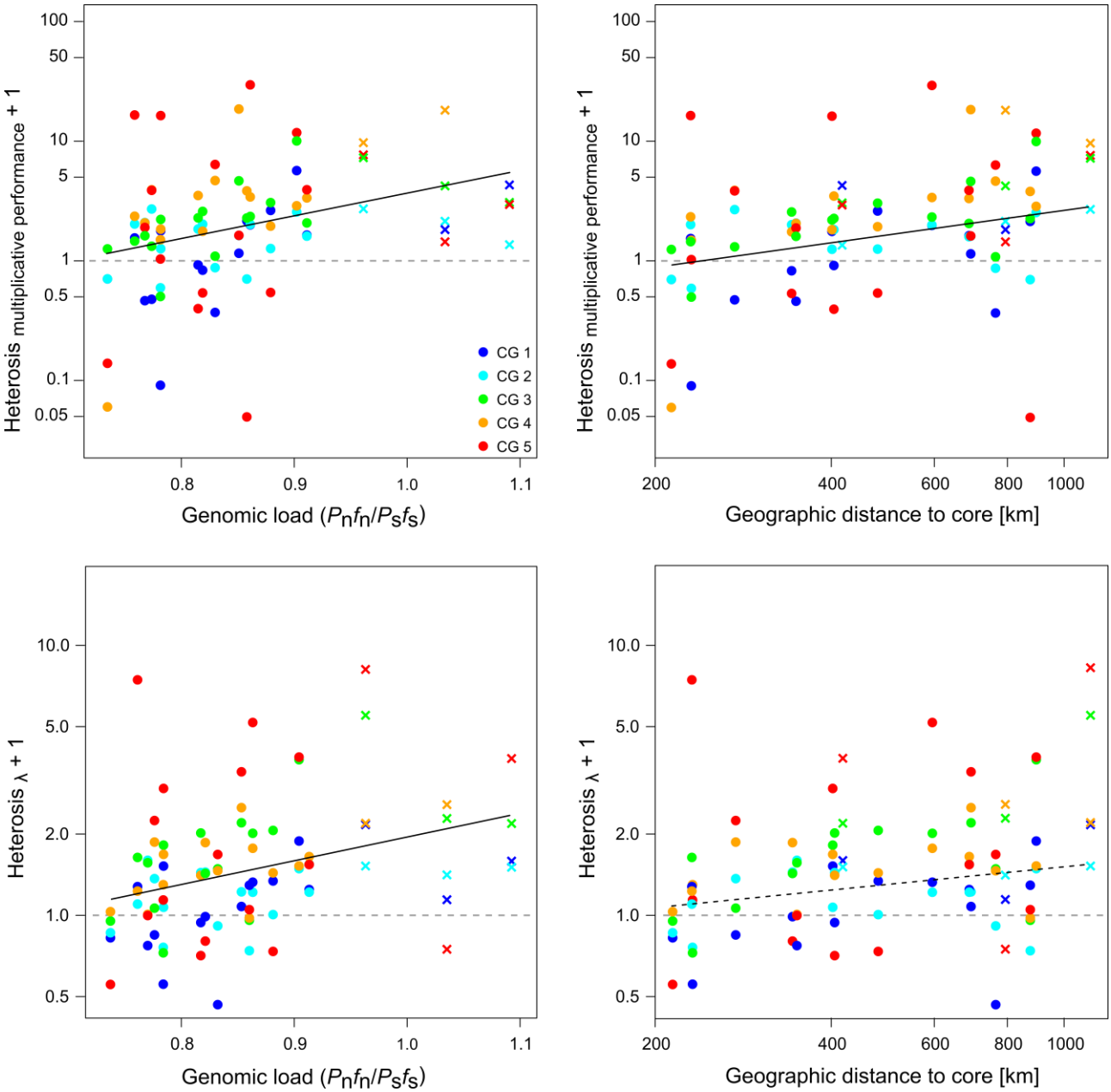

Figure 2: Relationship between heterosis in multiplicative performance or demographic rate and a genomic estimate of mutational load or geographic distance to core. Heterosis was estimated based on *multiplicative performance I* up to year 3 (**top**) or on λ (**bottom**) at the population level within each common garden site (CG1-5). Outcrossing populations are indicated by dots, selfing populations are indicated by crosses. Black lines represent the significant (full) or marginal (dashed) model-predicted slopes for heterosis (from test statistics in Table 1). The gray dashed line represents the value at which heterosis drops below 0, indicating outbreeding depression. Genomic estimate of mutational load (genomic load, **left**) was the ratio of genome-wide non-synonymous polymorphic sites multiplied by their derived mean frequency to synonymous polymorphic sites multiplied by their derived mean frequency. Geographic distance to core (**right**) was the distance of a population back to the glacial refugium along the map-projected population phylogeny, or the direct great-circle distance to the glacial refugium for older populations. Test statistics are reported in Table 1 and Table S10.

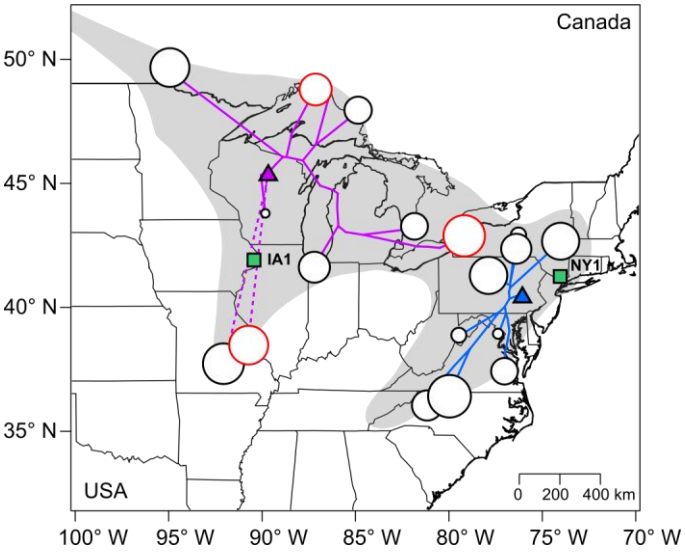

Figure 3: Expressed mutational load estimated by heterosis is increased at range edges of *Arabidopsis lyrata*. The 18 populations studied are represented by dots of varying diameter, porportional to their $\log_{10}$-transformed mean heterosis across common garden sites, calculated based on multiplicative performance including flower production during two reproductive seasons ($\log_{10}(\text{heterosis}_{\text{multiplicative performance I to Y3}} + 1)$ ranging from -1.4 to 1.4). Solid lines in purple and blue indicate the map-projected phylogeny from the western and eastern cores (presumed glacial refuge areas indicated by trianges) or connections to the core for older populations in the southwest (dashed purple lines). Mating system of populations is indicated by circle color: black for outcrossing and red for selfing. The two populations used as pollon donors in between-population crosses are represented by green squares. The approximate range of the species is shown by the gray-shaded area.

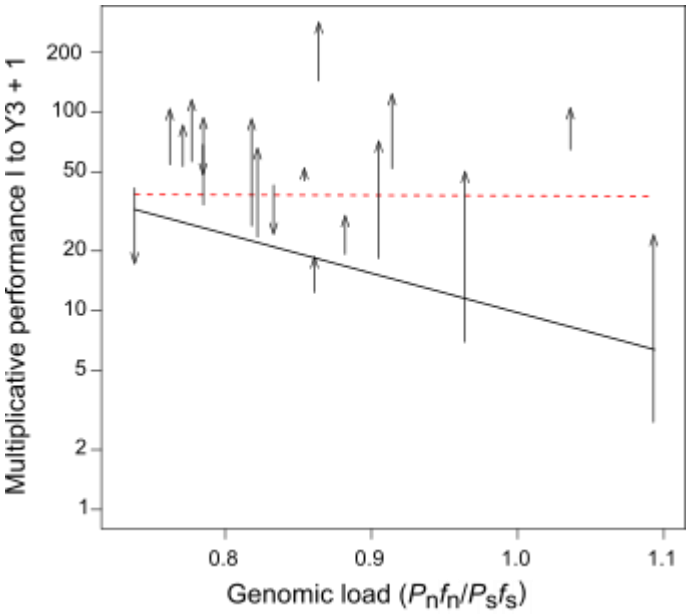

Figure 4: Relationshisp between population mean performance of within- (WPC) or between-population crosses (BPC) and the genomic estimate of mutational load. Lines represent model-predicted slopes for population mean *multiplicative performance I* up to year 3 of WPC (solid black) and BPC (dashed red) (from test statistics in Table S13). Genomic estimate of mutational load was the ratio of the genome-wide number of non-synonymous polymorphic sites multiplied by their mean derived frequency to the number of synonymous polymorphic sites multiplied by their mean derived frequency ($P_n f_n / P_s f_s$). Arrows represent the direction of change in mean performance across common garden sites from WPC (tail of the arrow) to BPC (head of the arrow). Fifteen out of 18 populations have arrows pointing upward, indicating heterosis.

Supporting information

Table S1: Information on the *Arabidopsis lyrata* populations studied

Population code	Latitude [° N]	Longitude [° W]	Ecological variables		Variables on population history				
			Min. temp. early spring [°C] †	Mean prec. summer [mm] †	Cluster‡	Mating system §	Genomic load ($P_n f_n / P_s f_s$) ‡	Distance to core [km]‡	F_{IS} §
IA1	41.97	90.37	-6.65	106.3	West	outcrossing	0.8069	402.03	0.065
IN1	41.61	87.19	-4.60	97.3	West	outcrossing	0.83283	761.43	-0.031
MD2	38.99	77.25	-1.75	95.3	East	outcrossing	0.78476	230.85	0.017
MO1	37.72	92.06	-2.40	94.3	West	outcrossing	0.90542	893.01	0.09
MO2	38.47	90.71	-2.65	88.0	West	selfing	1.03637	791	0.677
NC2	36.04	81.16	-1.65	118.3	East	outcrossing	0.91391	686.11	0.043
NC4	36.41	79.96	0.20	104.0	East	outcrossing	0.8641	593.27	0.021
NY1	41.30	73.98	-3.90	100.0	East	outcrossing	0.77297	193.11	0.051
NY4	42.35	76.39	-7.25	94.0	East	outcrossing	0.77737	273.62	0.011
NY5	42.66	74.02	-8.45	97.3	East	outcrossing	0.78503	400.86	0.094
NY6	43.00	76.09	-7.25	93.3	East	outcrossing	0.77053	348.12	-0.047
ON1	42.87	79.18	-6.10	82.3	West	selfing	0.96393	1105.49	0.7
ON11	48.77	87.13	-15.60	81.7	West	selfing	1.0927	417.24	0.95
ON12	49.65	94.92	-16.75	84.7	West	outcrossing	0.85352	690.89	0.025
ON3	43.26	81.84	-6.80	79.7	West	outcrossing	0.86054	872.34	-0.041
ON8	47.93	84.85	-14.70	84.0	West	outcrossing	0.88166	479.45	-0.038
PA3	41.28	77.87	-5.60	101.0	East	outcrossing	0.7618	230.24	0.02
VA1	37.42	77.02	0.35	108.0	East	outcrossing	0.81838	403.9	0.026
WI1	43.83	89.72	-10.00	98.7	West	outcrossing	0.73834	213.46	0.033
WV1	38.96	79.29	-3.70	96.0	East	outcrossing	0.82191	342.15	-0.052

† Data extracted from WorldClim database version 1.4 (Hijmans 2005). ‡ Willi et al. 2018. § Griffin and Willi, 2014

Table S2: Growth conditions during the crossing experiment

Growth Phase	Duration [days]	Temperature daytime [°C]	Temperature nighttime [°C]	Day length [h]	Light intensity [µmol m^{-2} s^{-1}]
Stratification	12	4	4	0	0
Germination	22	20	20	8	100
Growth †	21	22	20	10	140
Flowering initiation	10	22	20	16	240
Flowering and crossing	205	22	20	16	240

† Day length and light intensity were gradually increased every three days by 1h and 20 µmol m^{-2} s^{-1}, respectively.

Table S3: Summary of the crossing experiment and number of seeds sown in each common garden

Mother population	Father population	No. of cross families	No. of seeds sown in each common garden					Cross type
			CG1	CG2	CG3	CG4	CG5	
NY1	NY1	9	70	69	63	69	66	WPC
NY5	NY5	12	72	72	72	72	72	WPC
IN1	IN1	11	72	72	72	72	72	WPC
MO1	MO1	12	72	72	69	72	72	WPC
ON11	ON11	12	72	69	66	72	66	WPC
ON12	ON12	12	69	69	72	72	72	WPC
MO2	MO2	12	72	72	72	72	72	WPC
NC2	NC2	12	69	70	72	72	72	WPC
NC4	NC4	11	72	72	72	72	72	WPC
IA1	IA1	9	70	72	57	72	66	WPC
VA1	VA1	9	72	72	72	72	72	WPC
MD2	MD2	10	69	69	69	72	69	WPC
WV1	WV1	12	69	69	66	72	60	WPC
PA3	PA3	11	72	72	72	72	72	WPC
NY6	NY6	12	72	72	66	72	63	WPC
NY4	NY4	10	70	69	69	72	72	WPC
WI1	WI1	10	66	72	72	72	72	WPC
ON8	ON8	6	27	30	27	37	27	WPC
ON3	ON3	8	69	69	69	72	66	WPC
ON1	ON1	12	72	72	72	72	69	WPC
NY5	NY1	12	72	72	72	72	72	BPC
IN1	IA1	11	72	69	69	72	72	BPC
MO1	IA1	12	72	72	72	72	72	BPC
ON11	IA1	9	55	54	54	*0*	72	BPC
ON12	IA1	10	72	72	72	72	72	BPC
MO2	IA1	12	72	72	72	72	72	BPC
NC2	NY1	12	69	70	69	69	72	BPC
NC4	NY1	10	72	72	72	72	72	BPC
VA1	NY1	10	72	72	72	78	72	BPC
MD2	NY1	9	72	72	72	72	72	BPC
WV1	NY1	11	72	72	66	72	66	BPC
PA3	NY1	11	72	72	72	72	72	BPC
NY6	NY1	11	72	72	72	72	72	BPC
NY4	NY1	11	72	72	72	72	72	BPC
WI1	IA1	11	78	72	72	72	72	BPC
ON8	IA1	5	34	35	33	39	33	BPC
ON3	IA1	10	63	63	63	72	63	BPC
ON1	IA1	12	72	72	72	72	69	BPC

Total number of seeds sown: 12,933

Total number of cross combinations: 401

Table S4: Information on common garden sites

Transplant site	Location	Latitude [° N]	Longitude [° W]	Min. temperatures early spring (°C) †
CG1 (NY)	Beyond northern edge	44.51	74.02	-5.65
CG2 (MA)	Northern edge	42.72	73.22	-2.40
CG3 (VA)	Center	38.43	78.86	1.60
CG4 (NC)	Southern edge	36.13	80.28	4.90
CG5 (GA)	Beyond southern edge	33.93	83.36	6.95
Mean northern pop. ‡	North	-	-	-2.13
Mean center pop. ‡	Center	-	-	1.01
Mean southern pop. ‡	South	-	-	3.98

† Data extracted from WorldClim database version 1.4 (Hijmans 2005); ‡ Data measured for mean population of the eastern cluster. Northern populations: NY4, NY5, NY6; center populations: PA3, MD2, WV1; southern populations: VA1, NC2, NC4.

Table S5 Description of performance estimates

Performance estimate	Type	Level	Description			Analysis
Multiplicative performance (MP)						
MP I to year 3	continuous	Pot	Germination rate * reproductive output 2018 + 2019		†	MCMC
MP II to year 3	continuous	Pot	Germination rate * number of fruits 2018 + 2019		†	MCMC
MP I to year 2	continuous	Pot	Germination rate * reproductive output 2018		†	MCMC
MP II to year 2	continuous	Pot	Germination rate * number of fruits 2018		†	MCMC
Life stage components			*From*	*To*		
Germination	binary	Seed	Day 0	31 days after sowing	‡	REML
Survival fall year 1	binary	Seed	31 days after sowing	Soil temp. <5 °C (fall 2017)	*§	REML
Survival winter year 1	binary	Pot	Soil temp. <5 °C (fall 2017)	Soil temp. >10 °C (spring 2018)	*§	REML
Survival summer year 2	binary	Pot	Soil temp. >10 °C (spring 2018)	Soil temp. <5 °C (fall 2018)	*	REML
Survival winter year 2	binary	Pot	Soil temp. <5 °C (Fall 2018)	Count of reproductive output (spring 2019)	*†	REML
Reproduction year 2						
Survival to flowering year 2	binary	Pot	Plants that survived from end of winter 2017/18 to flowering 2018			REML
Bolting	binary	Pot	Plants that survived to flowering and produced inflorescences or not			REML
Reproductive output	continuous	Pot	Sum of all flower organs for plants that bolted			REML
Number of fruits	continuous	Pot	Potential total number of fruits from plants that produced at least one flower			REML

* Soil temperature was monitored every hour over the whole length of the experiment by 5 iButton® (Maxim Integrated, San Jose, CA, USA) per common garden site, buried under 5 cm of substrate in an empty pot.

† except for CG3: no survival after assessment of reproductive output 2018

‡ except for CG1: second cohort in spring 2018

§ except for CG4: experiment restarted in December 2017

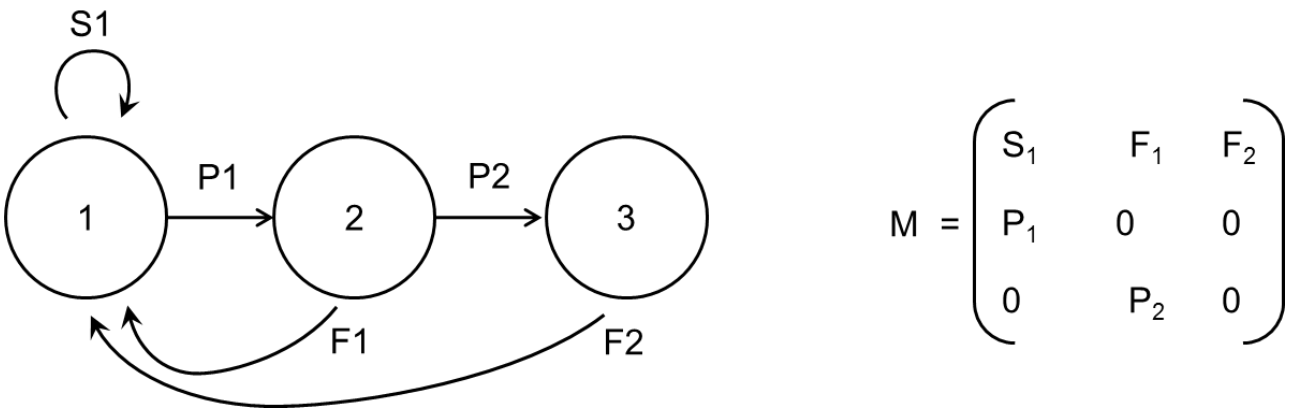

Figure S6: Estimation of population growth rate

For each combination of population, cross type and common garden site, a stage-classified matrix (**right**) was constructed based on assumptions about the life cycle (**left**). The life cycle was composed of three stages: 1–healthy seeds, 2–individuals capable of reproducing in the second year, 3–individuals capable of reproducing in the third year. The projection interval was set to one year for each stage. Survival between stage 1 and 2 (*P1*) was estimated as: germination rate in 2017 x survival from the seedling stage until the date of first flowering in the first reproductive period (year 2) at each site. Survival between stage 2 and 3 (*P2*) was estimated as the survival from the date of first flowering in the first reproductive period to the date of recording of reproductive output in the second reproductive period (year 3). We assumed that seeds that did not germinate in the first year (graduation from step 1 to 2) could survive over winter and contribute to the seed pool of the next years. We defined the probability to remain at the same stage (*S1*) as the survival of seeds over one winter. This estimate was calculated based on the seed burial experiment over one winter. The probability to remain at the same stage was set to 0 for both stage 2 and 3, assuming that no plants survived after the third year. Preliminary analysis showed that allowing individuals to remain in stage 3 indefinitely with the same probability of surviving each year than between stage 2 and 3 did not significantly affect the population growth rates (data not shown). Fecundity of stage 2 and 3 (*F1* and *F2* respectively) were estimated for each stage separately as: probability to reproduce * number of fruits * number of healthy seeds per fruit. While an estimate of the latter could have come from the crossing experiment, we assumed that these values were not reflective of the natural conditions and could introduce too much bias. Furthermore, fecundity of both stages would need to be adjusted for the chance of landing in a suitable environment for germination (including environmental effects, inter- and intraspecific competition, predation, etc.). We therefore decided to assign to all populations a standard value representing both number of healthy seeds per fruit and the probability to land in an environment suitable for germination, estimated as the value that yielded an average λ of 1 across all WPC over all sites.

Appendix S7: Parametrization of the hierarchical mixed-effects models

S7A: Hierarchical mixed-effects model with population heterosis as dependent variable

Model = lmer($\log_{10}$(heterosis + 1) ~ genomic load + (1 | maternal population) + (1 | common garden),
data = data)

S7B: Priors, and hierarchical mixed-effects model analyzed in a Bayesian (MCMC) framework, with individual multiplicative performance as dependent variable

Priors

Priors were set to be weak, using parameter expansion to improve convergence. *R* specifies the priors for the fixed effects, *G* specifies the priors for the random effects.

```
priors.model=list(
  R=list(V=diag(2), n=1, fix = 2),
  G=list(G1=list(V=diag(2), n=2, alpha.mu = rep(0,2),alpha.V = diag(2)*25^2),
       G2=list(V=diag(4), n=4, alpha.mu = rep(0,4),alpha.V = diag(4)*25^2),
       G3=list(V=diag(2), n=2, alpha.mu = rep(0,2),alpha.V = diag(2)*25^2),
       G4=list(V=diag(4), n=4, alpha.mu = rep(0,4),alpha.V = diag(4)*25^2),
       G5=list(V=diag(2), n=2, alpha.mu = rep(0,2),alpha.V = diag(2)*25^2),
       G6=list(V=diag(2), n=2, alpha.mu = rep(0,2),alpha.V = diag(2)*25^2)))
```

Parametrization of hierarchical mixed-effects models analyzed in a Bayesian (MCMC) framework

Multiplicative performance estimates were split into two datasets: the *zero_part*, a binary transformation of performance estimates with *zero_part* = 1 if performance > 0, else *zero_part* = 0; and the *norm_part* containing only the performance measures if *zero_part* = 1.

```
model = MCMCglmm(cbind(norm_part, zero_part) ~ trait -1 + trait:cross type * trait:genomic load,
                 random = ~ us(trait):maternal population
                 + us(trait:cross type):maternal population
                 + us(trait): maternal population: maternal family
                 + us(trait: cross type):maternal population:maternal family
                 + us(trait):common garden + us(trait):common garden:block,
                 prior = priors.model,
                 rcov = ~idh(trait):units,
                 family=c('gaussian', 'categorical'),
                 burnin = 5000, thin = 100, nitt = 50000,
                 data=data)
```

S7C: Hierarchical mixed-effects model with individual performance estimate as dependent variable

Dependent variable with binary distribution

Model = glmer(performance ~ cross type * genomic load
+ (1 + cross type | maternal population / maternal family)
+ (1 | common garden / block),
family = "binomial",
data = data)

Dependent variable with log-normal distribution

Model = lmer($\log_{10}$(performance + 1) ~ cross type * genomic load
+ (1 + cross type | maternal population / maternal family)
+ (1 | common garden / block),
data = data)

Table S8: Summary of individual performance, and heterosis based on population mean performance (in % for binary variables) up to three years at five common garden sites

	Individual performance		Population heterosis			
Dependent variable	***N***	**Mean**	***N***	**Min.**	**Mean**	**Max.**
Multiplicative performance (MP)						
MP I to year 3	6703	58.50	89	-0.96	1.88	23.50
MP II to year 3	6703	36.67	89	-0.92	2.65	30.23
MP I to year 2	6703	37.54	89	-0.95	2.96	41.21
MP II to year 2	6703	26.27	89	-0.97	3.89	71.45
Life stage components						
Germination	12933	64.2 %	89	-0.68	0.01	0.75
Survival fall year 1	7214	77.6 %	89	-0.40	0.10	1.75
Survival winter year 1	5072	85.2 %	89	-0.28	0.07	0.48
Survival summer year 2	4319	29.8 %	89	-0.83	0.64	12.42
Survival winter year 2	608	61.8 %	44	-0.68	0.11	5.00
Reproduction year 2						
Survival to flowering year 2	3731	99.6 %	89	-0.25	0.17	2.46
Bolting	3719	60.2 %	89	-0.53	0.57	5.42
Reproductive output †	2240	134.72	89	-0.82	0.69	8.00
Number of fruits	2218	96.09	82	-0.84	0.74	8.98
Fertilization rate ‡	2240	67.2 %	82	-0.47	0.09	0.76
Demographic rate						
λ	189	1.18	89	-0.53	0.73	7.29

† Sum of buds, flowers, fruits and pedicels produced by one individual
‡ Not analyzed

Table S9: Magnitude of effect of the genomic estimate of mutational load or geographic distance to core and mating system (F_{IS}) on (log_{10}-transformed) heterosis, and magnitude of effect of the genomic estimate of mutational load on (log_{10}-transformed) multiplicative performance (MP) of within-population crosses (WPC)

	Increase in heterosis + 1 (x-fold)				*Decrease in WPC performance (%)*	
Dependent variable	***N***	**Genomic load**	**Distance to core**	F_{IS}	***N***	**Genomic load**
MP I to year 3	89	4.7 *(1.0; 4.6)*	3.4 *(0.9; 3.0)*	*NS*	189	-80.3 *(32.6; 6.4)*
MP II to year 3	89	5.6 *(1.1; 6.1)*	3.4 *(1.0; 3.6)*	*NS*	189	-73.3 *(19.8; 5.3)*
MP I to year 2	89	7.5 *(1.0; 7.2)*	3.1 *(1.1; 3.3)*	3.2 *(1.5; 4.8)*	189	-75.7 *(21.0; 5.1)*
MP II to year 2	89	7.9 *(1.0; 8.0)*	3.2 *(1.1; 3.6)*	3.3 *(1.6; 5.3)*	189	-69.5 (*14.4; 4.4)*
λ	89	1.3 *(1.06; 1.36)*	*NS*	1.7 *(1.0; 1.7)*	-	-

For the general models of Table 1 analyzing heterosis, the magnitude of effect of a predictor variable was calculated as: ratio between the back-transformed predicted heterosis corresponding to the maximal value of a predictor variable, compared to the back-transformed predicted heterosis corresponding to the minimal value of a predictor variable. As in some cases outbreeding depression (negative heterosis values) was observed, 1 was added to predicted heterosis as outbreeding depression can take the maximal value of -1. For the general model of Table S13 analyzing WPC, the magnitude of effect of genomic load was calculated as: percentage difference between the back-transformed predicted performance corresponding to the maximal value of the predictor variable (in parenthesis, right) in our sampling and the back-transformed predicted performance corresponding to the minimal value of the predictor variable (in parenthesis, left).

Table S10: Summary of models testing for the effect of geographic distance to core on heterosis of outcrossing populations only, estimated on population mean performance estimates up to three years at five common garden sites

		Distance to core					
Dependent variable	***N***	***Estimate***	***χ²***		***R²m***	***R²c***	
Multiplicative performance (MP)							
MP I to year 3	75	**0.75**	**4.69**	*	0.096	0.289	†,§
MP II to year 3	75	**0.76**	**5.43**	*	0.101	0.373	†
MP I to year 2	75	0.65	3.75	(*)	0.080	0.331	§
MP II to year 2	75	**0.68**	**4.26**	*	0.081	0.341	
Life stage components							
Germination	75	0.10	1.90		0.033	0.455	
Survival fall year 1	75	0.02	0.19		0.002	0.243	§
Survival winter year 1	75	0.01	0.14		0.002	0.040	‡
Survival summer year 2	75	0.10	0.42		0.005	0.132	
Survival winter year 2	38	-0.23	1.63		0.042	0.042	†
Reproduction year 2							
Survival to flowering year 2	75	-0.01	0.13		0.001	0.249	
Bolting	75	0.08	0.70		0.007	0.310	
Reproductive output	70	**0.45**	**4.08**	*	0.097	0.286	‡,§
Number of fruits	70	**0.43**	**4.57**	*	0.096	0.235	‡

Population heterosis estimates (log_{10}-transformed) were assumed to follow Gaussian distributions. Test statistics include the regression coefficient (*estimate*), χ^2-value, and the marginal and conditional R^2 of the model. Distance to core was standardized prior to analyses (mean = 0). Model fits with significant (positive) intercept are indicated by ‡. Estimates with P-values < 0.05 are written in bold; significance is indicated: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. The *bobyqa* optimizer was used when models initially failed to converge (§). Results for random effects are not shown. For one of the five common gardens (CG3), the experiment stopped early and variables consider performance to year 2 only (indicated by †).

Table S11A: Summary of models performed on the level of individual pots, testing for the effect of cross type (between- compared to within-population crosses [0]), genomic estimate of mutational load and their interaction on multiplicative performance (MP) based either on flower production (MP I) or fruit production (MP II) up to one or two reproductive seasons, at five common garden sites

		Log-normal process, fixed effects									
		Cross type			**Genomic load**			**Cross type * genomic load**			
Dependent variable	***N***	***Mean***	***HPD interval***		***Mean***	***HPD interval***		***Mean***	***HPD interval***		
MP I to year 3	6703	**0.134**	(0.021,0.229)	*	-0.708	(-1.465,0.113)	(*)	**0.995**	(0.252,1.774)	***	†,‡
MP II to year 3	6703	**0.143**	(0.049,0.243)	**	-0.517	(-1.308,0.241)		**0.844**	(0.148,1.574)	***	†,‡
MP I to year 2	6703	**0.121**	(0.026,0.222)	*	-0.635	(-1.415,0.137)		**0.87**	(0.081,1.573)	***	‡
MP II to year 2	6703	**0.135**	(0.04,0.233)	**	-0.494	(-1.202,0.283)		**0.82**	(0.044,1.533)	***	‡

		Logistic process, fixed effects									
		Cross type			**Genomic load**			**Cross type * genomic load**			
Dependent variable	***N***	***Mean***	***HPD interval***		***Mean***	***HPD interval***		***Mean***	***HPD interval***		
MP I to year 3	6703	**0.491**	(0.226,0.754)	***	-0.972	(-3.438,1.803)		1.568	(-0.446,3.644)		†
MP II to year 3	6703	**0.497**	(0.241,0.742)	***	-1.15	(-3.713,1.447)		1.756	(-0.103,3.610)	(*)	†
MP I to year 2	6703	**0.471**	(0.223,0.761)	***	-1.134	(-3.788,1.713)		1.594	(-0.492,3.641)		
MP II to year 2	6703	**0.462**	(0.221,0.703)	***	-1.172	(-3.835,1.321)		1.632	(-0.262,3.432)	(*)	

Multiplicative performance estimates (log_{10}-transformed if >0) were assumed to follow Gaussian distributions with 0-inflation. Therefore, models assessed all fixed and random effects for their importance in both the Gaussian process (total number of flowers or fruits produced during one or two reproductive seasons) and the logistic process (binary variable depicting germination combined with survival and the capacity to initiate flowering). Estimates of coefficients are modes of an MCMC sample from the posterior distribution of parameters (mean and higher posterior density, *HPD* interval). The logistic part of the model predicts non-zeros in the distribution on the logit scale. Genomic load was standardized prior to analyses (mean = 0). Model fits with significant (positive) intercept are indicated by ‡. Estimates with *P*-values < 0.05 are written in bold; significance is indicated: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Results for random effects are not shown. For one of the five common gardens (CG3), the experiment stopped early and variables consider performance to year 2 only (indicated by †).

Table S11B: Summary of mixed-effects models performed on the level of individual pots, testing for the effect of cross type (between- compared to within-population crosses [0]), genomic estimate of mutational load and their interaction on performance based on germination and survival up to three years at five common garden sites, and reproduction in the second year

		Cross type (CT)			Genomic load			CT * genomic load					
Dependent variable	***N***	***Estimate***	χ^2		***Estimate***	χ^2		***Estimate***	χ^2		***R^2m***	***R^2c***	
Life stage components													
Germination	12933	0.07	0.32		0.42	0.08		-1.15	0.71		0.001	0.260	
Survival fall year 1	7214	**0.22**	**11.78**	***	-1.10	1.34		0.23	0.10		0.003	0.237	‡
Survival winter year 1	5072	**0.47**	**22.40**	***	-0.53	0.34		0.92	0.66		0.006	0.297	‡,§
Survival summer year 2	4319	**0.51**	**17.40**	***	-0.96	0.40		1.57	1.29		0.008	0.552	§
Survival winter year 2	608	-0.16	0.60		1.60	0.63		-0.11	0.00		0.003	0.227	†,‡,§
Reproduction year 2													
Survival to flowering year 2	3731	0.18	2.96	(*)	0.22	0.04		1.30	1.10		0.002	0.404	‡
Bolting	3719	**0.51**	**14.62**	***	**-4.49**	**8.25**	**	**3.00**	**3.97**	*	0.019	0.453	
Reproductive output	2240	**0.12**	**5.75**	*	-1.01	3.81	(*)	0.99	3.04	(*)	0.018	0.314	‡,§
Number of fruits	2218	**0.14**	**7.88**	**	-0.73	2.04		0.85	2.24		0.014	0.306	‡,§

Germination, survival and bolting were binary variables; the respective models predict non-zeros on the logit scale. All other performance estimates were $\log_{10}$-transformed and assumed to follow Gaussian distributions. Test statistics include regression coefficients of each fixed effect (*estimate*), χ^2-values, and the marginal and conditional R^2 of the model. Genomic load was standardized prior to analyses (mean = 0). Model fits with significant (positive) intercept are indicated by ‡. Estimates with *P*-values < 0.05 are written in bold; significance is indicated: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. The *bobyqa* optimizer was used when models initially failed to converge (§). Results for random effects are not shown. For one of the five common gardens (CG3), the experiment stopped early and variables consider performance to year 2 only (indicated by †).

Table S12: Summary of models performed on the level of population means, testing for the effect of cross type (between- compared to within-population crosses [0]), genomic estimate of mutational load and their interaction on mean performance up to three years (per cross type) at five common garden sites

		Cross type (CT)			Genomic load			CT * genomic load				
Dependent variable	***N***	***Estimate***	χ^2		***Estimate***	χ^2		***Estimate***	χ^2		***R^2m***	***R^2c***
Multiplicative performance (MP)												
MP I to year 3	189	**0.29**	**12.75**	***	**-1.99**	**5.20**	*	**1.91**	**4.57**	*	0.097	0.571 †,‡
MP II to year 3	189	**0.31**	**16.03**	***	**-1.62**	**3.92**	*	1.62	3.79	(*)	0.096	0.606 †,†
MP I to year 2	189	**0.28**	**15.35**	***	**-1.73**	**5.76**	*	**1.71**	**4.70**	*	0.086	0.685 ‡
MP II to year 2	189	**0.28**	**17.03**	***	**-1.45**	**4.56**	*	**1.54**	**4.31**	*	0.083	0.692 ‡
Life stage components												
Germination	189	0.01	0.75		0.01	0.02		-0.10	1.61		0.007	0.806
Survival fall year 1	189	**0.01**	**8.66**	**	-0.08	3.19	(*)	0.05	1.53		0.015	0.829 ‡
Survival winter year 1	189	**0.01**	**13.59**	***	0.02	0.60		0.00	0.02		0.029	0.642 ‡,§
Survival summer year 2	189	**0.02**	**6.93**	**	0.02	0.11		0.04	0.28		0.006	0.909 ‡,§
Survival winter year 2	189	-0.01	0.31		-0.10	0.62		0.15	0.74		0.008	0.317 †,‡,§
Reproduction year 2												
Survival to flowering year 2	189	0.00	0.67		0.01	0.22		0.03	1.68		0.007	0.807 ‡
Bolting	189	**0.02**	**11.31**	***	**-0.19**	**10.12**	**	**0.15**	**5.44**	*	0.021	0.862 ‡
Reproductive output	189	**0.11**	**4.00**	*	-1.00	3.79	(*)	**1.32**	**4.69**	*	0.041	0.651 ‡,§
Number of fruits	189	**0.13**	**6.03**	*	-0.75	1.84		1.15	3.69	(*)	0.038	0.672 ‡,§

Population mean performance estimates for each cross type (log_{10}-transformed) were assumed to follow Gaussian distributions. Test statistics include regression coefficients of each fixed effect (*estimate*), χ^2-values, and the marginal and conditional R^2 of the model. Genomic load was standardized prior to analyses (mean = 0). Model fits with significant (positive) intercept are indicated by ‡. Estimates with *P*-values < 0.05 are written in bold; significance is indicated: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. The *bobyqa* optimizer was used when models initially failed to converge (§). Results for random effects are not shown. For one of the five common gardens (CG3), the experiment stopped early and variables consider performance to year 2 only (indicated by †).

Table S13: Summary of models testing for the effect of genomic estimate of mutational load on population mean performance up to three years of within- (WPC) and between-population crosses (BPC) separately, at five common garden sites

		Genomic load						Genomic load			
Dependent variable	***N* (WPC)**	***Estimate***	χ^2	R^2m	R^2c		***N* (BPC)**	***Estimate***	χ^2	R^2m	R^2c	
Multiplicative performance (MP)												
MP I to year 3	100	**-1.99**	**5.20** *	0.082	0.575	†,‡	89	-0.02	0.00	0.000	0.552	†,‡
MP II to year 3	100	**-1.62**	**3.92** *	0.060	0.622	†,‡	89	0.04	0.00	0.000	0.568	†,‡
MP I to year 2	100	**-1.73**	**5.76** *	0.060	0.704	‡	89	-0.01	0.00	0.000	0.652	‡
MP II to year 2	100	**-1.45**	**4.56** *	0.047	0.718	‡	89	0.09	0.01	0.000	0.649	‡
Life stage components												
Germination	100	0.01	0.02	0.785	0.784		89	-0.08	0.78	0.817	0.827	
Survival fall year 1	100	-0.08	3.38 (*)	0.825	0.836	‡	89	-0.02	0.33	0.824	0.824	‡
Survival winter year 1	100	0.02	0.47	0.603	0.621	‡,§	89	0.02	1.15	0.699	0.715	‡,§
Survival summer year 2	100	0.02	0.11	0.889	0.865	‡,§	89	0.06	2.68	0.923	0.920	‡,§
Survival winter year 2	100	-0.25	0.27	0.232	0.232	†,‡,§	89	0.05	0.22	0.431	0.472	†‡,§
Reproduction year 2												
Survival to flowering year 2	100	0.01	0.26	0.782	0.782	‡	89	0.04	3.67 (*)	0.799	0.807	‡
Bolting	100	**-0.19**	**10.45** **	0.870	0.875	‡	89	-0.02	0.20	0.856	0.866	‡
Reproductive output	100	-1.00	3.72 (*)	0.045	0.625	‡,§	89	0.27	0.26	0.004	0.647	‡,§
Number of fruits	100	-0.75	1.84	0.023	0.670	‡,§	89	0.37	0.55	0.007	0.646	‡,§

Population mean performance estimates for each cross type ($\log_{10}$-transformed) were assumed to follow Gaussian distributions. Test statistics include regression coefficient (*estimate*), χ^2-value, and the marginal and conditional R^2 of the model. Genomic load was standardized prior to analyses (mean = 0). Model fits with significant (positive) intercept are indicated by ‡. Estimates with P-values < 0.05 are written in bold; significance is indicated: (*) P<0.1, * P<0.05, ** P<0.01, *** P<0.001. The *bobyqa* optimizer was used when models initially failed to converge (§). Results for random effects are not shown. For one of the five common gardens (CG3), the experiment stopped early and variables consider performance to year 2 only (indicated by †).

Chapter 3: **Decrease in pollination service from northern to southern range limits in the North American plant *Arabidopsis lyrata***

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Abstract

Climate factors have attracted a lot of attention in the study of species distribution limits, while little is known about the role of biotic interactions. They may also contribute to the establishment of the range limits as they change together with climate over space, or may interact together in affecting the distribution of the species. Here, we monitored insect pollinators using time-lapse cameras in populations of the North American plant *Arabidopsis lyrata* over a transect spanning from the southern to the northern range limit. With approximately 4500 hours of observation, we tested whether pollinator services declined from the core to the edge of species distribution and what the driving factors were: low plant census size, low flower density, less attractive flowers due to marginal conditions, fewer flowering plants species, and/or unfavourable temperature conditions. Overall, we spotted 67 pollinating insect taxa, supporting the idea that the plant-pollinator network is a generalist system. Interestingly, pollination services declined from the core to the southern range edge but not at the northern range populations. None of the hypothesized mechanisms for declining pollination service was supported. However, we found that the chance of a flower being visited by a pollinator generally increased with plant census size, and that visitation rate per flower decreased in high-density patches. Although mechanisms remain elusive, the strong decline in pollination service toward the southern range limit suggests that for an herbaceous plant of the temperature zone, low pollination services could be involved in the contribution of the establishment at the southern range limits.

Introduction

Species range limits, when not caused by dispersal limitation, are a reflection of species niche limits. Ecological factors that seem often limiting species ranges are climatic such as temperature and precipitation (Sexton *et al*. 2009). Even though the field of species distribution modeling (SDM) has suggested that a handful of climate variables can often explain distribution limits rather well (e.g., Normand *et al.* 2009; Lee-Yaw *et al*. 2016), the contribution of biotic interactions has been rarely considered in distribution modeling, and in the study of species distribution limits more generally (Sexton *et al*. 2009). Even though this field has been poorly explored, theoretical and empirical studies have shown that antagonistic interactions affect species persistence, including interspecific competition (Jankowski *et al*. 2010; Stanton-Geddes *et al*. 2012), parasites and pathogens (Briers 2003; Coates *et al*. 2017), and herbivores (Galen 1990; Benning *et al*. 2019). Similarly, evidence was found for the role of mutualistic interactions affecting the persistence of both species involved such as nitrogen-fixing bacteria (Stanton-Geddes & Anderson 2011) and flower-insect interactions (Stone & Jenkins 2008; Chalcoff *et al*. 2012; Moeller *et al*. 2012). Even though the literature on biotic interactions and their effect at range limits has been increasing, it is still ambiguous whether these factors contribute in range stablishment as they normally vary temporally in accordance to abiotic conditions.

Pollination services can be especially important for sexual reproduction and therefore for the population dynamics as four-fifths of temperate-zone flowering plant species rely on animal pollinators for reproduction success (Ollerton *et al*. 2011). Limited pollination services at range edges might be relevant for many plant species that rely on insects as pollen vectors for reproduction (Gaston 2009). For example, a meta-analysis of transplant experiments has shown that plant reproduction declined beyond the range for 73% of the species considered (Hargreaves *et al*. 2014). If the decline in reproduction is not only due to climatic conditions

but also because of a lack of potential pollinators to transfer pollen, range limits might be also constrained by a lack of pollination services (Moeller *et al*. 2012). The variation in pollination services across the species distribution gradient is often related to the climatic conditions that favour the activity of the pollinators (Herrera 1990; Moeller *et al*. 2012). However, other factors could reveal why marginal populations might experience poor pollination services. For example, population census size and flower density, the richness of flowering plant species or the adverse climatic conditions toward the range edge might contribute in explaining the differences in pollination across the gradient. Below we discuss these potential mechanisms and their relation with species range limits affecting pollination services.

A first mechanism is based on the observation that across the distribution of species, abundance tends to decline toward the range edges. The hypothesis for a pattern of declining abundance was explained by a decline in habitat availability from the core to the margins of species distribution (Brown 1984). The so-called abundant-center hypothesis has been generally supported in a recent meta-study, documenting a decline of both the density of individuals and the density of populations from the core to the edges of species distribution (Pironon *et al*. 2017). If marginal plant populations consist of fragmented patches of lower density or have lower census size, this may affect negatively the presence of pollinators (e.g., Kunin 1997; Stone & Jenkins 2008; Elliott & Irwin 2009). A likely reason is that pollinators exhibit a preference for patches with a high local or regional density (Ohashi & Yahara 1999). If this is the case, this could lead to the situation of an Allee effect (Courchamp *et al*. 1999), where pollination service is reduced in small populations or populations with low local plant densities at species range edges.

A second mechanism may be related to reduced attractiveness at the range edges due to resource limitation. As animal-pollinated plants tend to evolve characteristics to maximize the efficacy of the transfer of pollen from plant to plant, a strategy to intensify attractiveness could

rely on increasing the number of flowers per plant and/or produce larger flowers, both of which have been shown to generally increase visitation rates (e.g., Klinkhamer & De Jong 1990; Grindeland *et al.* 2005). However, investments in the floral display may be costly and if the environment is marginal, the resources may be limited (Stone & Jenkins 2008). Furthermore, range edge populations seem often affected by long-time exposure to genetic drift due to past expansion or long-term isolation, mutation accumulation, and reduced population mean performance (Willi *et al*. 2018; Willi & Van Buskirk 2019) that may affect flower attractiveness. Moreover, if pollination services are chronically low, a transition from outcrossing to selfing might occur at range edge populations (Morgan & Wilson 2005; Moeller 2006). Range edge populations are associated with high self-compatibility rates (e.g., Griffin & Willi 2014), presumably accompanied by changes in floral morphology such as a reduction in flower size (Darling *et al*. 2008; Dart *et al*. 2012). Hence, reduced attractiveness of flowers at species range edges may have ecological and genetic motives.

A third mechanism may be related to the richness of flowering plant species and the diversity of resources offered to pollinators. Studies reported a positive relationship between the diversity of flower types among plant species and the diversity and abundance of pollinators (e.g. Biesmeijer *et al*. 2006; Lázaro & Totland 2010). The richness and abundance of other flowering plants increase the diversity of resources available for pollinators and therefore attract a broader diversity of insect visitors. However, if the conditions at the edge of a species range become marginal for many other plant species and the plant community is less diverse, these areas might have less diversity and abundance of pollinators.

A fourth mechanism for reduced pollination service at a plant's range edge may be related to the adverse climatic conditions for pollinator activity. As ecological conditions are expected to become harsher toward the edges, guilds of pollinators that are to some extent specialized in a community of plants maybe also decline in abundance. It is well known that

pollinator abundance and metabolic activity are highly affected by temperature and elevation (Stone & Jekins 2008; Hillyer & Silman 2010; Chalcoff *et al.* 2012; Moeller *et al.* 2012). It is reasonable to think that if there is an environmental gradient that limits plant populations, a similar gradient could have consequences for the pollinator assembly (e.g., Battisti *et al*. 2006).

In this study, we tested whether pollination services decreased toward the edges of a plant species' range (research question I) and the contribution of the five mechanisms to any decrease (research question II). Our study organism was the short-lived perennial plant *Arabidopsis lyrata* subsp. *lyrata* in North America, which has been the subject of ongoing research focused on the ecological and evolutionary aspects of his distribution range (see Lee-Yaw *et al*. 2018; Willi *et al*. 2018; Perrier *et al*. 2020; Sánchez-Castro *et al*. 2020). We assessed *visitation rate* and *pollination rate* in 13 populations across a latitudinal gradient of 1100km along the eastern United States including replicate populations at the southern and northern range limit. We quantified and identified pollinators by the use of time-lapse cameras in each population, and related data to population and site characteristics.

Material and methods

Study organism

Arabidopsis lyrata represents two subspecies with a circumpolar arctic-alpine distribution: *A. lyrata* subsp. *petraea* of central and northern Eurasia, and *A. lyrata* subsp. *lyrata* of central and eastern North America (Schmickl *et al.* 2010). North American *A. lyrata* (from now on abbreviated as *A. lyrata*) has a well-defined distribution separated in two distinct ancestral clusters in the eastern US and Canada, from North Carolina to the state of New York, and in the Midwest, from Missouri to south-western Ontario (Willi & Määttänen 2010; Willi *et al.* 2018). The species is mostly outcrossing and insect-pollinated, however, it has been shown that some populations at the range edges are self-compatible and predominantly selfing (Griffin &

Willi 2014). A total of 13 populations were selected and monitored on a latitudinal gradient of 1.100km from North Carolina to upstate New York. Populations were categorized based on their geographical position within the range in *southern range edge*, *center*, and *northern range edge* (Fig. 1A, Table S1). Plants produce basal rosettes with inflorescences emerging from their centers during the blooming period (Fig. 1B), which takes place from mid-April to mid-June for eastern populations. The number of flowers varies depending on the population and plant age. They produce nectar discs at the base of the anthers and volatile compounds to attract pollinators (Peer & Murphy 2003). Populations are normally found on sand dunes and rocky outcrops, although they can also occur on sandy or rocky riverbanks and on rocky shorelines. Along the Appalachians, plants normally grow on poor soils made of coniferous leaf litter and moss on the top of the raw rock, and under evergreen trees dominated by Virginia pine (*Pinus virginiana)* and the Eastern Red Cedar (*Juniperus virginiana)* that create a bit of shield.

Pollination records

Personal observations of pollinators in the field can lead to misinterpretation because of the short observation periods (Rafferty & Ives 2011; Hargreaves *et al*. 2015), the effect of the observer on insect behaviour (Peckham & Peckham 1905), and the difficulties to carry live identifications. As an alternative and based on Edwards *et al*. (2015) research, we used time-lapse cameras to record pollinators (TLC 200 Pro HDR, Brinno, Taipei City, Taiwan, Fig. 1C). Time-lapse cameras take images at short intervals over time, capturing complete records over the entire day and allows to monitor simultaneously several patches of the same habitat. The cameras provide enough precision to identify and quantify flower visitors independent of the flower or insect type (Edwards *et al*. 2015). At each selected population, 10 to 12 cameras recorded separate flower patches for approximately 3 days during the daylight for a 12-hour period, from 8 am to 8 pm (see Table S1 for detailed observation period and patches recorded).

We did not conduct nocturnal observations as flowers close after sunset. As abundance of insect visitors is highly affected by temperature, wind, and precipitation (Cruden 1972; Roubik 1989, p. 98), observations were carried when the weather was sunny and the sky was clear. We followed Edwards *et al*. (2015) protocol in setting the time-lapse cameras to take images every 3 seconds. They had demonstrated that 90% of total visits were captured as insects generally stayed longer than 3 seconds on the flowers. The observation period was carried during the time of full bloom, from mid-April in the south to the beginning of June in the north, for two consecutive years, in 2018 and 2019. Although repeated records would be ideal, we were not able to monitor all populations in both years.

Videos were examined with the *Quick Time Player* program (Apple, California, US), which allowed us to select the captions when a visit was observed. Visits were considered only if there was direct contact of the insect with the pistil or stamens of the flower. We discarded from the analysis members of the Formicidae (ants) as their contribution as pollinators have been shown to be ineffective (Junker *et al*. 2007). Also, members of Curculionidae (weevil beetles) were not considered due to the complexity to identify and quantify them. Additionally, the genus *Meligethes* within Coleoptera was not considered as a pollinator but as a ravenous flower herbivore. Therefore, infected flowers were discarded from the analysis. For each patch and day, only mature and fully open flowers in the video frame were considered. As the number of flowers recorded varied between patches within and between populations, we corrected for the abundance of flowers observed in the video frame calculating daily *visitation rate*. First, the total number of insect-flower interactions depicted by each camera per day were counted (considered as abundance), which was divided by the total number of open flowers in the video frame, known as *visitation rate* (n° visits $flower^{-1}$ day^{-1}). Additionally, we calculated a second variable, defined as the ratio of flowers visited at least once during the course of a day and the total number of flowers observed in the video frame, called *pollination rate* (visited $observed^{-1}$

flowers^{-1} day^{-1}). The complete sample size was: 13 populations x 1-2 years of recording per population x 10-12 cameras per population and year x 2-4 days of recording per camera in a population and year = 382.

Even though not all flower visitors act as pollinators and those who do also vary in their effectiveness in pollinating, we counted all visits equally. Each observed visit in the video was saved as an image for insect identification, together with information on the flower visited (each flower had a unique ID per day and camera), the time of the day, and the temperature when the interaction occurred. We identified insects to the lowest taxonomic unit given the quality of pictures, using Kits *et al*. (2008); Miranda *et al*. (2013); Skevington *et al*. (2019) as identification keys. If the images where too blurred and the pollinator was not recognizable, the visit was categorized as "unidentifiable". Importantly, not all visits were identified to the same taxonomic depth. Some groups were split into morphotypes based on main phenotypic characters such as morphological features, size, and color patterns – especially in the Hymenoptera group. The total number of different taxa/morphotypes observed was defined as *pollinator richness*. We also calculated Simpson (1949) and Shannon (1948) *biodiversity indices* based on pollinator abundance and *richness* at the level of camera and day.

Population and site characteristics

We quantified several plant population characteristics related to the hypothesized mechanisms of pollination service decline toward range limits. *Plant census size* was calculated by multiplying the area with occurrence of *A. lyrata* with the average density of plants. Plant occurrence was estimated by calculating the area and recording surfaces with presence and absence of plants. Local plant density was estimated at each patch where a camera was set up, as the total number of flowering plants per m^2. *Local flower density* was the total number of

open *A. lyrata* flowers on the m^2 at the patches; it was considered an estimate of local availability of flowers to pollinators. We assessed floral size on 40 randomly chosen, mature flowers, each of a different plant, in each population. *Flower size* was estimated by measuring and multiplying the length of the ovary and the maximal width of the corolla. Measurements were done during mid-day when flowers were fully open. Finally, *flowering plant species richness* was estimated, as the total number of flowering plant species co-occurring temporally and spatially with *A. lyrata*. To assess the effect of temperature at each side of the range, two data loggers (DS1922L, Maxim *iButton*, CA, USA) collected air temperature hourly at each population while cameras were recording.

In summary, *plant census size*, *flower size*, and *plant species richness* were variables estimated on the level of an *A. lyrata* population in a year; while *daily mean temperature* and *local flower density* were variables estimated on the level of a patch of *A. lyrata* that was monitored in a population and year.

Statistical analysis

All main analyses were performed on daily *visitation rate* as a dependent variable. To test whether pollinator service declined from the centre of *A. lyrata* distribution toward the range limits (research question I), we performed a first analysis based on generalised linear mixed-effects model (GLMM) in the R environment (R Core Team 2019). Secondary variables such as *pollination rate*, *pollinator richness*, and *diversity indices* (Shannon and Simpson) were also considered. Fixed effect was range position (*southern range edge*, *centre*, and *northern range edge*), with the reference being the *centre*. Random effects were daily replicate in a population and year, and population. Mechanistic variables were also analyzed for a relationship with range position, i.e. *population census size* (log_{10}-transformed), *local flower density* (log_{10}-transformed), *flower size*, *plant species richness*, and daily *mean temperature*. All these

variables were analyzed individually with restricted maximum likelihood, with the R package lme4 (Bates *et al*. 2015) and LmerTest (Kuznetsova *et al.* 2017).

In the second part of the analyses, we investigated the factors hypothesized to influence *visitation rate* (research question II). The main dependent variable was again daily *visitation rate*, analyzed by GLMM. Secondary testing included the other variables depicting pollination services: *pollination rate*, *pollinator richness*, and *diversity indices* (Shannon and Simpson). Fixed effects were the mechanistic variables of *population census size* (log_{10}-transformed), *local flower density* (log_{10}-transformed), *flower size*, *plant species richness*, and daily *mean temperature*. These predictors were mean-centered. Random effects were daily replicate in a population and year, and population.

Results

The total observation period for all populations including ech camera and day was 4.522 hours. During this recording period, 7.310 flowers were monitored, and 17.508 insects were spotted on *A. lyrata* flowers. Out of the total visits, we were able to identify 88% of them to the order level. About 48.9% of the identified insects were Hymenoptera of the Apocrita group, followed by 47.7% Diptera. Lepidoptera represented only 3.2% of the visits and Coleoptera 0.1% (Table S2). The fraction of each insect order varied among populations without a clear pattern differentiating between the southern, center, and northern range position (Fig. 2A). In the Diptera, Syrphidae and Bombyliidae were the most frequent visitors of *A. lyrata* (46% and 32% respectively), followed by Muscoidea and Empididae (Table S3). Southern *A. lyrata* populations were visited more often by bombyliids; while centre and northern populations were visited more frequently by syrphids (Fig 2B). Some of the taxa were observed in more than one population, particularly the hoverfly *Toxomerus,* which was spotted as a common visitor in all populations. Although populations had some insect taxa that overlapped across populations,

there were also unique pollinators at each location. We found in total 67 different morphotypes pollinating *A. lyrata* along the 1100 km gradient (see Table S4 for the taxonomic list).

I. Do pollination services decline from the core toward range edges?

A first model tested for the effect of population position across the range of *A. lyrata* distribution on daily *visitation rate* per flower (Table 1; mean values and standard errors for each population in Table S5). *Visitation rate* was significantly lower in southern-range populations compared to centre and northern. While in some populations flowers had less than a visit per day, the populations of NC2 and VA2, other populations were frequently visited with more than five interactions per flower and day, in NY4 and WV1 populations (Table S5). Figure 3A shows the daily *visitation rate* based on populations means for each position in the range, illustrating that southern range populations had a significantly lower number of interactions than centre and northern populations.

A similar trend was observed for *pollination rate*, where southern populations had a lower fraction of flowers visited per day compared to centre and northern populations (Table 1, Fig. 3B). In some populations, less than half of the observed flowers were visited at least once (MD1, VA2), while in others, the *pollination rate* reached more than 80% (MD4, NY4, see Table S5). Moreover, no significant differences were observed for pollinator richness and biodiversity indices with population range position (Table 1).

Among the mechanistic variables, only a few trends were observed with the range position (Table 1). The total *population census size* did not differ between range position for the 13 populations of *A. lyrata* selected. Some populations occupied a very small range distribution area and had a small population census size –such as MD1, NC1, VA; while others had a greater extension not necessarily coinciding with centre populations (see Table S7). We found differences in *flower size* out of the 520 flowers measured in the field, where the two

populations monitored in Virginia, southern range, had the greatest size (VA1, and VA2, see Table S6 and Fig. S1A). Overall, there was a trend that *flower size* was slightly greater in southern-range populations (Table 1). The richness of flowering *plant species* showed a trend of increase from centre toward northern sites – the total number of flowering plant species coinciding in time and space with *A. lyrata* is listed in Table S8.

The variation observed between cameras and days within the same population in daily *visitation rate* and *pollination rate* is shown in Figure S1, and is the subject of another study which analyzes in a finer detail the variation of the pollination network across scales (Sánchez-Castro *et al*. 2020 in prep.). Figure S.2 shows the correlation matrix between the different pollination services variables (Fig S2.A), and the correlation among the predictors (Fig. S2.B). Pollination services seemed to be highly correlated, especially *visitation rate* and *pollination rate*, but also *pollination richness* and both biodiversity indexes. However, no correlation was found between these potential mechanistic factors.

II. What are the mechanisms for reduced pollination services?

Despite the fact that mechanistic variables predicted to explain reduced *visitation rates* toward range limits were not associated with range limits, we tested for an association with pollinators independent of the range position of *A. lyrata* (research question II). Results are shown in Table 2. *Visitation rate* was negatively correlated with *local flower density*, indicating that the dayily visitation rate per flower decreased when the density of flower was high (Fig. 4). No other of the mechanistic variables were correlated with *visitation rate*. However, *pollination rate* was significantly higher in larger *A. lyrata* populations. As figure 5A shows, those northern and centre populations that were larger, indicated by the size of the bubble, had a higher *pollination rate*, indicated by brightness colours. Figure 5B represents also the distribution map of the populations selected, the *population census size*, and the relationship with *visitation rate*. This

indicates that the rate of flowers visited per day was higher at those populations with a greater number of individual in the population

Analyses on further variables depicting pollinator diversity revealed an important role of flower density (Table 2). Pollinator diversity was higher when there were more flowers in a patch. Furthermore, larger flowers attracted or tended to attract a more diverse community of pollinators. Ultimately, results did not show a correlation between the *richness of flowering plant species* neither with *visitation rate* of pollinator richness.

Discussion

Range limits and species distribution models have mainly focused on the abiotic ecological factors as the main causes to understand geographic range limits. However, here we have shown that there is no reason to assume that biotic interactions are less relevant contributing to or at least stabilizing range margins. Pollination services in *A. lyrata* were significantly lower at the southern edge, most likely contributing to that range limit. The testing of mechanistic hypotheses did not provide clear support for why this should happen. However, results indicated the potential importance of low *population census size* affecting the chance that a flower is visited. We discuss these and other results in the context of species range limits and pollination biology in general.

Pollination services at the range limits

Based on climate data of the recent past, environment niche modeling has been shown that range limits reflect niche limits for *A. lyrata* (Lee-Yaw *et al*. 2018). A similar conclusion was drawn in a recent transplant experiment with sites beyond the species range in south and north confirming that southern range limits reflect niche limits as survival, reproduction, and

population growth declined, constraining the species persistence toward the southern range (Sánchez-Castro *et al.* 2020 in prep). In the transplant experiment, the main cause of performance decline seemed climatic. The results found here indicate that pollination services were significantly reduced in natural populations at the southern range limit for *A. lyrata* (Fig. 3); however, the development of siliques indicated that pollinators at the common garden sites in the south did not seem to be lacking. This indicates that reduced *visitation rate* may not be a primary source causing range limits, but it may contribute to the reduced reproduction and small population size if pollination services are chronically low. A pollen limitation study (supplemental pollen to receptive flowers and assessment of the seed set) would have been ideal to have a better understanding whether range edge populations with low pollination services suffer also from pollen limitation and seed set. Due to time and resource limitations, the assessment of this aspect was not doable in this study. The discrepant results found by the transplant experiment and the niche modeling for the northern range were interpreted in the context of warmer conditions in the north caused by global warming. Here we found that both *visitation rate* and *pollination rate* per flower were not different between populations of *A. lyrata* of center and north. The result found here motivates that on the side of pollination, *A. lyrata* should not be limited in tracking climate warming. More limiting than pollination seems the distribution of habitat toward the north, as the species would have to penetrate areas dominated by boreal forests.

The role of pollinators as stabilizing factor of range limits was found previously in other studies. For example, it has been shown that populations of *Witheringia solanacea* in Costa Rica had greater visitation and fruit set in the lower montane site compared to the upper elevational limit (Stone & Jenkins 2008). Similar conclusions were found for *Embothrium coccineum* in Northwestern Patagonia, where populations had lower pollination services at the eastern range limit, directly related to water stress (Chalcoff *et al*. 2012). Also for *Clarkia*

xantiana in the Sierra Nevada, where the abundance and visitation rates of pollinators decreased and pollen limitation increased at the range limits compared to core populations (Moeller *et al*. 2012). On the contrary, there was no evidence that pollination activity decreased at the upper range limit for *Rhinanthus minor* in the Rocky Mountains (Hargreaves *et al*. 2015). Apart from these studies, the role of pollinators stablishing the range edge remains unexplored. Moreover, the contribution of biotic factors driving adaptation remains uncertain due to their fluctuation in regards to climatic factors. Two recent meta-analysis studies tested for the importance of considering biotic interactions in transplant experiments on local adaptation patterns, finding no evidence of adaptation to biotic factors but they did affect the fitness of the organisms (Hargreaves *et al.* 2020; Briscoe *et al.* 2020).

Interestingly, none of the mechanistic hypotheses for reduced pollination services at the southern edge of species distribution were supported. No evidence for deterioration of conditions toward range edges was found, except for a trend of greater flower size toward the south. However, *pollination rate* was lower in *A. lyrata* populations of small census size. Furthermore, *visitation rate* decreased within increasing *local flower density*. Despite neither census size decreased toward the southern range limit nor flower density of patches increased, they may still play a role in reducing pollination service. Potential causes or stabilizers of range limits may rarely be consistently important across all range-edge populations. In previous project, we found that range edges were associated also with enhanced genetic drift due to past range expansion or rear-edge isolation (Willi *et al*. 2018), reduced population mean fitness due to mutation accumulation (Perrier *et al*. 2020) and constrained local adaptation (Sánchez-Castro *et al.* 2020 submited). It is probably the adding of additional factors, such as pollination constrains that in the end cause the pattern of distribution limits that we observe. In this sense, our results clearly support a contribution by reduced pollination services to range limits. But results about the drivers made also clear that one or several other factors that we ignored might

explain the decline of insect visitation rates toward the southern range limit. Interestingly, our results on flower size has shown a trend where southern populations had greater flower size than centre populations coinciding with highest inbreeding coefficients rates (Griffin & Willi 2014). This contradicts previous findings where changes in floral morphology as a reduction in size and herkogamy have been suggested as a common phenomenon in marginal populations that switched to selfing reproduction (Darling *et al*. 2008; Dart *et al*. 2012).

Pollination biology of A. lyrata

A recent study on one *A. lyrata* population in Isle Royale pointed to Syrphids as main pollinators, in particular the genus *Toxomerus* (Edwards *et al*. 2019). By extending the geographical range of the study, we found that both Hymenoptera and Diptera were equally important as main pollinators, while Lepidoptera represented a small proportion of the visits (Fig. 2A). In the Diptera, hoverflies were the most frequent family in the centre and northern populations, supporting the previous results of Edwards *et al*. (2009), while Bombyliidae predominated at lower latitudes (Fig. 2B). Even though we found some common pollinators in all populations such as *Toxomerus*, most flowers were visited by multiple insect taxa. Findings support that many pollinators of the temperate zone are opportunists with labile preferences of pollen and nectar, demonstrating that the pollination network is a generalist system which provides ecological flexibility in terms of reproduction for the plant, and a diversity of food resources for the pollinator (Waser *et al*. 1996; Fenster *et al*. 2004). As the pollinator network is generalist for this particular species, lower chances for Allee effect as a consequence of declines in visitation when plant numbers are low would occur to species-specific pollinator interactions. However, Allee effect could still happen where the abundance of the insect community is small. The generalist network not only offers a greater flexibility for reproduction but also contributes to enhancing the fruit and seed set. Albrecht *et al*. (2012) showed in a

manipulated experiment with *Raphanus sativus* that the richness of the pollinators increased significantly the fruit and seed set production. Similar results were previously found by Fontaine *et al*. (2006). This does not apply only for flower-pollinator interactions, also with plants or bacterial communities, where the diversity of organisms facilitate a more heterogeneous niche of resources (Dimitrakopoulos & Schmid 2004).

What are the mechanisms for reduced pollination services?

Our results revealed a negative correlation between *flower density* and daily *visitation rate* per flower (Fig. 4). Results seem to point to a possible Allee effect. When flower density is low, few pollinators are attracted to a patch, but when the flower density is too high, the rate of visitation per flower declines. Some studies also found this negative correlation between flower density and pollinator abundance (Kunin 1993; Delmas *et al.* 2016). But positive correlations were also found (Grindeland *et al*. 2005; Nielsen & Ims 2000), or not even a relationship (Hendrickson *et al*. 2018). Higher flower density seems to maintain a higher diversity of pollinators. Additionally, the positive correlation between *pollination rate* and *population census size*; suggests that larger populations have also a greater fraction of flowers to be visited at least once. These results would support the idea that fragmented and small populations might attract fewer visitors lacking of pollinators to successfully complete plant reproduction (Stone & Jenkins 2008).

In regards to flowering plant species richness, we did not found a correlation with pollination services, indicating that the richness of flowering plant species did not increase the diversity of pollinators in the habitat. It is assumed that the diversity of floral resources is expected to increase visitation rate (Ghazoul 2006; Hegland & Boeke 2006) but also might attract a greater number of pollinator species (Lázaro & Totland 2010). Furthermore, we found that larger flowers attracted a higher pollinator richness, and as a trend, higher pollinator

diversity. An additional model testing for the correlation between flower size and the diversity of pollinators with latitude did not show any significant results, indicating that southern latitudes not always imply a greater diversity of species than northern latitudes as suggested by Schemske *et al*. (2009).

In summary, we have shown that pollination services vary across the distribution range, with an especially decrease at the southern range edge of *A. lyata*. The mechanistic variables used in this study did show evidence for a deterioration of the conditions towards the range edges. However, population size and flower density were significantly correlated with pollination services suggesting that bigger populations attract more pollinators per flower, while small populations had significantly fewer visitors which could potentially lead to Allee effect. Overall, the reinforcement of range limits via declines in visitation rates is a phenomenon shown recently in several studies, however, more research is needed to understand whether this occurs only in special cases or if it is a common phenomenon that happens in many other plant species. We encourage the importance of this study to the inclusion of biotic interactions in niche models for a more realistic prediction of the range and species distribution limits.

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Table 1. **Results of mixed-effects models testing foran association bewteenrange position of *Arabidopsis lyrata* populations and pollination services or mechanistic variables thougth to affect pollinators**

		Southern range			Northern range		
		Estimate		*SE*	*Estimate*		*SE*
Pollination services	N						
Visitation rate	382	**-1.670**	*	0.846	0.916		0.990
Pollination rate	382	-0.174	(*)	0.091	0.086		0.107
Pollinator richness	382	0.356		0.793	0.830		0.934
Shannon index	382	0.062		0.201	0.177		0.237
Simpson index	382	0.022		0.056	0.032		0.065
Mechanistic variables							
Pop census size ($\log_{10}$)	13	-0.539		0.519	0.646		0.614
Local flower density ($\log_{10}$)	166	0.118		0.240	-0.080		0.282
Flower size	520	7.704	(*)	4.071	7.341		4.817
Plant sp. richness	13	1.083		1.195	2.917	(*)	1.413
Mean T°	39	-0.435		2.281	-3.424		2.669

The effect of range position, southern and northern range, was compared with the range centre of *A. lyrata* distribution. The number of replicates (*N*) was the number of original observations, either per day for pollinator data, or on the level of population or camera recording at a patch for mechanistic variables. Estimates with *P*-values < 0.05 are written in bold; significance is indicated: (*) $P<0.1$, * $P<0.05$. Results for random effects are not shown.

Table 2. Results of mixed-effects models testing for an association bewteen population census size, the local flower density, flower size, flowering plant species richness, and daily mean temperature on pollination of *Arabidopsis lyrata* flowers

		Pop census size (log10)			Flower density (log10)			Flower size			Plant sp. richness		Mean T°	
		Estimate		*SE*	*Estimate*		*SE*	*Estimate*		*SE*	*Estimate*	*SE*	*Estimate*	*SE*
Pollination services	N													
Visitation rate	382	0.726		0.500	**-1.259**	**	0.469	0.014		0.064	0.337	0.217	0.004	0.072
Pollination rate	382	**0.115**	*	0.049	-0.010		0.042	0.002		0.006	0.019	0.021	-0.004	0.007
Pollinator richness	382	0.141		0.288	**0.916**	***	0.247	**0.090**	*	0.037	0.186	0.125	-0.003	0.045
Shannon index	382	0.031		0.076	**0.241**	***	0.070	0.019	(*)	0.010	0.046	0.033	-0.004	0.012
Simpson index	382	0.006		0.028	-0.016		0.043	0.003		0.004	0.003	0.012	-0.005	0.006

Visitation rate, pollination rate, pollinator richness, Shannon, and Simpson diversity indexes were continuous.All predictors were mean centered. Test statistics include regression coefficients of each fixed effect (*estimate*) and standard error values (*SE*). Coefficients are written in bold when $P< 0.05$. Significance is indicated: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. The *bobyqa* optimizer was used to help models to converge.

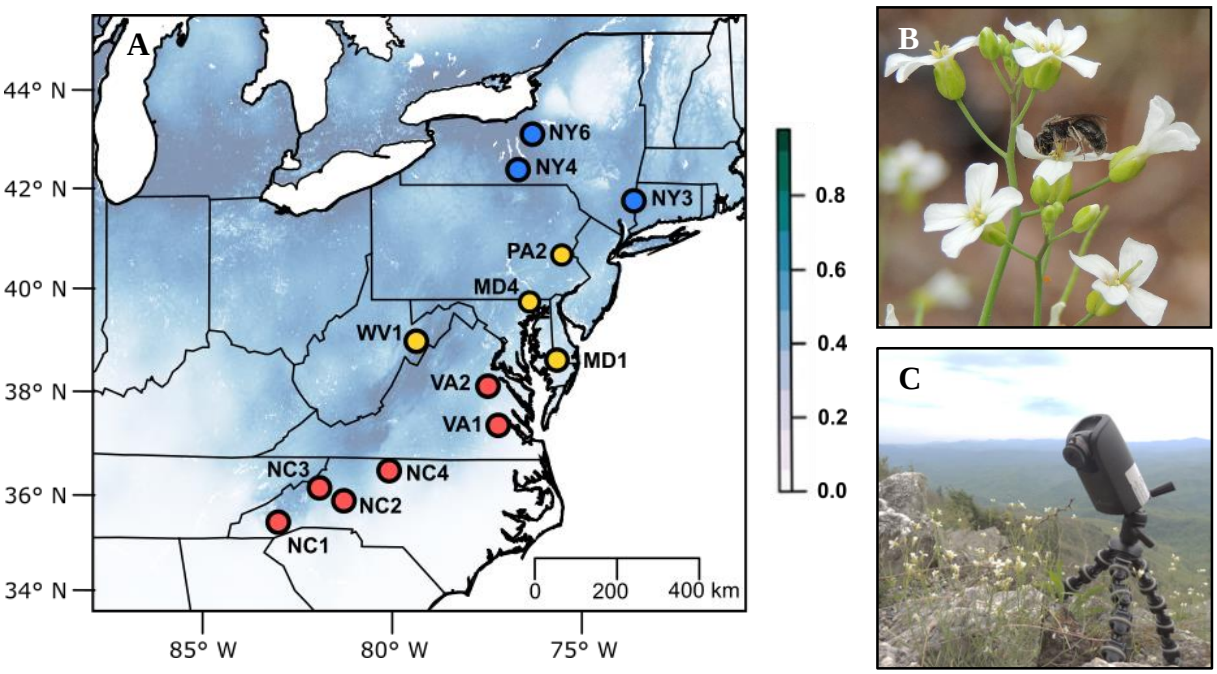

Figure 1. Distribution of the 13 *Arabidopsis lyrata* populations studied for pollination service in North America (A), an image of *A. lyrata* flowers with a wild bee visiting (B), and a time-lapse camera monitoring a patch of flowers in the field (C). Panel A shows populations indicated by dots accompanied by a three-digit abbreviation (Table S1, the two letters stand for the state in the US, the number for latitudinal position within state as in Willi *et al.* 2018). Red dots indicate populations of the southern range of distribution, those in yellow the center, and those in blue the northern range. Shades of blue indicate habitat suitability revealed by niche-modelling (Lee-Yaw *et al.* 2018). Panel B shows several flowers of *A.lyrata* pollinated by a wild bee. Panel C shows the time-lapse camera recording a patch of flowers from the same vantage point for several days.

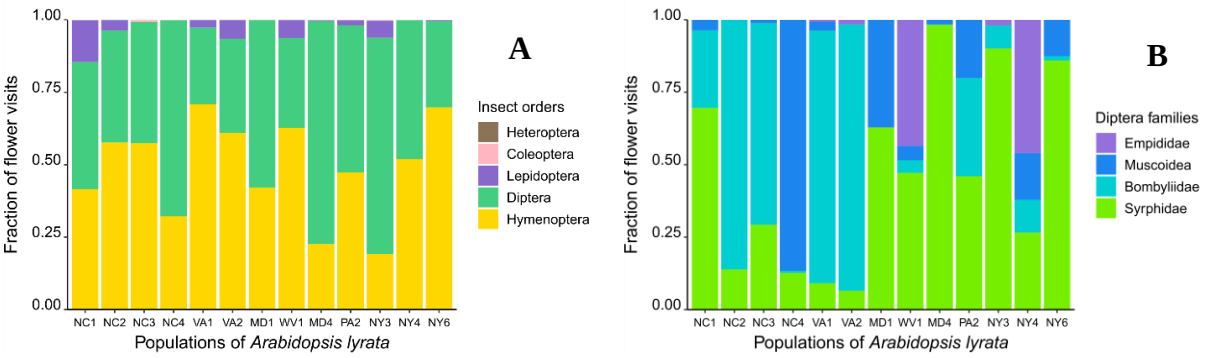

Figure 2. The fraction of insect orders (A) and Diptera families (B) that visited *Arabidopsis lyrata* flowers in the 13 populations, sorted from south (left of the x-axis) to north (right). For population abbreviations see Fig. 1.

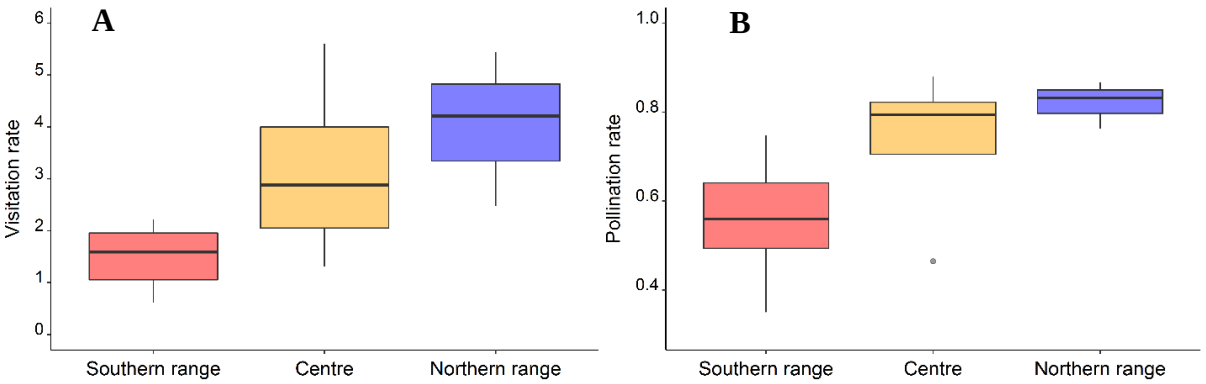

Figure 3. Insect visitation rate (A) and pollination rate (B) in *Arabidopsis lyrata* populations differing in range position, sorted from southern range to northern range (x-axis). Box plots were based on populations means, calculated based on patch means within population and year that were then averaged across the two years of observation. The thick line of each box represents the median, the coloured box the interquartile range, the whiskers the variability outside the upper and lower quartiles, and the individual dots the outliers.

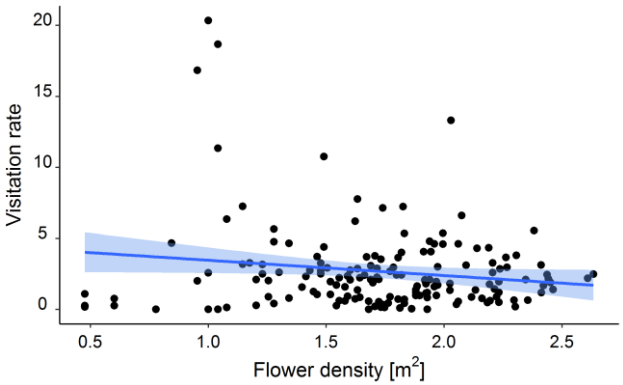

Figure 4. Visitation rate of pollinators in *Arabidopsis lyrata* depending on flower density. It shows the negative correlation between visitation rate based on daily replicates means per population and the density of flowers. The model-predicted regression line is shown in blue, with the lower and upper 95% confidence interval.

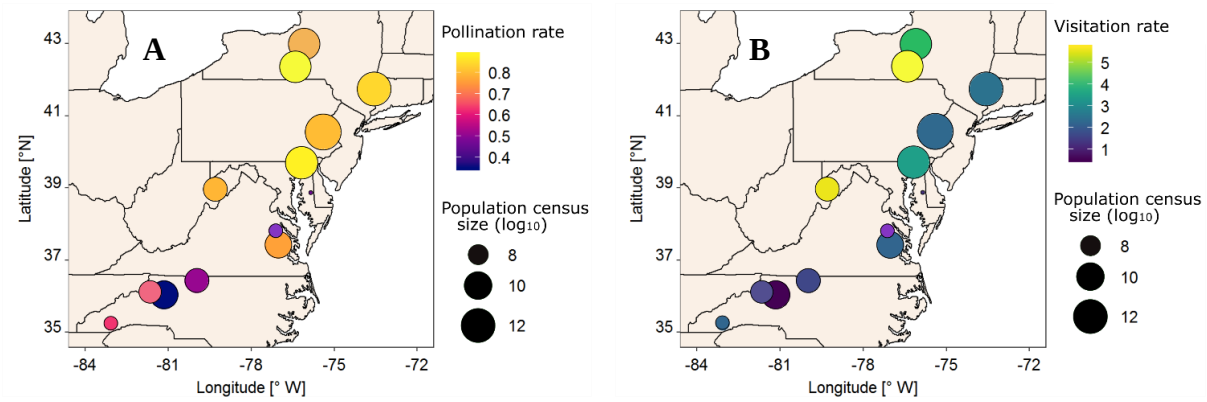

Figure 5. Map illustrating the relationship between pollination rate (A) and visitation rate (B) with population census size in the 13 populations of *Arabidopsis lyrata*. In Panels A and B, the size of circles represents the census size and the color the visitation rate per flower or the pollination rate of flowers. Population means for pollination were based on means per temporal replicated and then on cameras and finally on year.

Supporting information

Table S1. General information on the *Arabidopsis lyrata* populations studied

Pop	Location	Distribution	Latitude [° N]	Longitude [° W]	Elevation [m a.s.l.]	Area [m^2]	Days of recording	N° of patches	Total hours
MD1	Martinak St. Park	Centre	38.86	75.84	2	64	2	10	208
MD4	Conowingo	Centre	39.7	76.19	79	5,925	2	10	262
NC1	Tuckasegee	South	35.25	83.08	1,015	184	3.5	21	379
NC2	Moravian Falls	South	36.04	81.16	680	5,031	4	20	331
NC3	Blowing Rock	South	36.11	81.66	1,108	682	5.5	6	400
NC4	Mayodan	South	36.41	79.96	224	1,149	3	9	308
NY3	Dover Plains	North	41.73	73.56	143	10,264	3	12	397
NY4	Ithaca	North	42.35	76.39	442	3,222	3	12	424
NY6	Clark State Park	North	43	76.09	229	7,185	4	20	418
PA2	Allentown	Centre	40.57	75.4	125	8,743	3	12	400
VA1	Sandybottom	South	37.42	77.02	5	2,175	3	12	428
VA2	Aylett	South	37.81	77.12	10	110	3	8	260
WV1	Hopeville	Centre	38.96	79.29	395	1,001	3.5	10	307

Population abbreviation (ID), location, distribution in the range, latitude, longitude, elevation, surface area with occurrence, the number of days of recording pollinators; the number of patches in the population; and the total number of hours of recording.

Table S2. Order diversity rate of the main insect groups at each population

Fraction rate of the main insect orders				
Population	**Hymenoptera**	**Diptera**	**Lepidoptera**	**Coleoptera**
MD1	42.1	57.9	0.0	0.0
MD4	22.6	77.0	0.2	0.1
NC1	41.6	43.9	14.4	0.2
NC2	57.7	38.6	3.7	0.1
NC3	57.4	42.0	0.0	0.7
NC4	32.1	67.7	0.0	0.0
NY3	19.2	74.7	5.9	0.3
NY4	51.9	48.0	0.0	0.1
NY6	69.8	29.8	0.2	0.2
PA2	47.3	50.8	1.8	0.1
VA1	70.8	26.6	2.6	0.0
VA2	61.1	32.4	6.6	0.0
WV1	62.7	31.1	6.2	0.0
Average	48.9	47.7	3.2	0.1

Table S3. Diversity rate of the main diptera families at each population

Fraction rate of the main Diptera families				
Population	**Syrphidae**	**Bombyliidae**	**Muscoidea**	**Empididae**
MD1	62.9	0.0	37.1	0.0
MD4	98.3	0.1	1.6	0.0
NC1	69.6	26.8	3.6	0.0
NC2	13.9	86.1	0.0	0.0
NC3	29.3	69.6	1.1	0.0
NC4	12.7	0.7	86.7	0.0
NY3	90.1	7.9	0.0	1.9
NY4	26.6	11.3	16.2	46.0
NY6	86.0	1.5	12.5	0.0
PA2	46.0	33.9	20.1	0.0
VA1	9.1	87.2	3.0	0.7
VA2	6.5	91.9	0.0	1.6
WV1	47.2	4.3	5.0	43.5
Average	46.0	32.4	14.4	7.2

Table S4. List of insect species/morphotypes observed pollinating *Arabidopsis lyrata* sub. *lyrata* in North America

	Population												
Specie/ Morphotype	**MD 1**	**MD 4**	**NC 1**	**NC 2**	**NC 3**	**NC 4**	**NY 3**	**NY 4**	**NY 6**	**PA 2**	**VA 1**	**VA 2**	**WV 1**
Alipia octomaculata	0	0	0	0	0	0	0	0	0	1	0	0	0
Anthocharis midea	0	0	1	1	0	0	0	0	0	0	0	0	0
Apocrita morphotype 1	0	0	0	0	1	1	0	1	1	0	0	0	1
Apocrita morphotype 2	1	1	0	1	1	0	0	1	0	0	1	0	0
Apocrita morphotype 3	0	0	1	0	0	0	0	0	0	1	0	0	0
Apocrita morphotype 4	0	1	0	0	0	0	0	1	0	1	0	0	0
Apocrita morphotype 5	0	1	0	1	0	0	0	0	0	0	0	0	0
Apocrita morphotype 6	0	0	0	0	0	0	0	0	0	0	1	1	1
Apocrita morphotype 7	0	0	0	0	0	0	0	0	0	0	1	1	0
Apocrita morphotype 8	0	0	0	0	0	0	0	0	0	1	1	0	0
Apocrita morphotype 9	0	0	1	0	0	0	0	0	0	0	0	0	0
Apocrita morphotype 10	0	0	0	0	0	0	1	0	0	0	0	0	0
Apocrita morphotype 11	0	0	0	0	0	0	0	0	0	0	0	1	0
Apocrita morphotype 12	0	0	0	0	0	0	0	1	0	0	0	0	0
Apocrita morphotype 13	0	0	0	0	0	0	0	0	0	0	0	0	1
Apocrita morphotype 14	0	0	0	0	1	0	0	0	0	0	0	0	0
Apocrita morphotype 15	0	0	1	0	0	0	0	0	0	0	0	0	0
Asterocampa sp.	0	0	1	1	0	0	0	0	0	0	1	1	1
Bombus sp.	0	0	0	0	1	0	0	0	0	0	1	0	0
Bombylius major	0	1	1	1	1	1	1	1	1	1	1	1	0
Bombylius pulchellus	0	0	0	0	0	0	0	0	0	1	0	0	0
Bombylius pygmaeus	0	0	0	0	0	0	0	1	0	0	0	0	0
Callophrys grynaeus	0	0	0	0	0	0	1	0	0	1	0	0	0
Chloropidae	0	0	0	0	0	0	0	0	1	0	0	0	0
Chrysotoxum sp.	0	0	1	0	0	0	0	0	0	0	0	0	0
Coleoptera	0	0	0	0	0	0	0	0	1	0	0	0	0
Conopidae	0	0	0	0	0	0	0	1	0	0	0	0	0
Cupido comyntas	0	0	0	0	0	0	0	0	0	0	1	1	0
Empididae	0	0	0	0	0	0	0	1	0	0	0	0	0
Empis sp.1	0	0	0	0	0	0	1	1	0	0	0	0	0
Empis sp.2	0	0	0	0	0	0	0	0	0	0	0	0	1
Empis sp.3	0	0	0	0	1	0	0	0	0	0	0	1	0
Epalpus sp.1	0	0	0	0	0	0	0	1	0	0	0	0	0
Epalpus sp.2	1	0	0	0	0	0	0	0	0	0	0	0	0
Eristalis saxorum	0	0	0	0	0	0	0	0	0	1	1	0	0
Eupeodes sp.	0	0	0	0	0	0	0	0	0	0	0	0	1
Eurythmia sp.	0	0	0	0	0	0	0	0	0	0	0	0	1
Halictidae fam	0	1	1	1	1	1	0	1	1	0	1	1	0
Heliophilus fasciatus	0	0	0	0	0	0	1	0	0	0	0	0	0
Hemipenthes sp.	0	0	0	0	0	0	0	0	0	0	0	0	1
Heteroptera	0	0	0	0	0	0	0	0	0	0	1	0	0

Mallota bautias	0	0	0	0	0	0	0	0	0	1	0	0	0
Mesembrina sp.	0	1	1	0	1	0	0	1	1	0	0	0	0
Moth sp.1	0	0	1	0	0	0	0	0	0	0	0	0	0
Moth sp.2	0	0	0	0	0	0	1	0	0	0	0	0	0
Moth sp.3	0	0	0	0	0	0	0	0	1	0	0	0	0
Moth sp.4	0	0	0	0	0	0	0	0	1	0	0	0	0
Muscoidea sp.1	0	0	0	0	0	0	0	0	0	1	0	0	0
Muscoidea sp.2	0	0	1	0	0	0	1	1	1	0	1	0	0
Muscoidea sp.3	0	1	1	0	0	1	0	0	0	0	0	0	1
Nomada sp.1	0	1	0	0	0	0	0	1	1	0	0	0	0
Nomada sp.2	0	0	0	0	0	0	1	0	0	0	0	0	0
Phyciodes sp.	0	0	1	0	0	0	0	0	0	0	1	0	0
Platycheirus sp.	0	0	1	0	1	1	1	0	0	1	0	0	0
Pyrausta orphisalis	0	0	1	0	0	0	0	0	0	0	0	0	0
Sciomyzidae	0	0	0	0	0	0	0	0	0	0	1	0	0
Sericomyia sp.	0	0	0	0	0	0	1	0	0	1	0	0	0
Sphaerophoria sp.	0	1	0	0	0	0	0	1	0	0	0	0	1
Syrphidae sp.1	0	0	1	0	0	0	0	0	0	0	0	0	1
Syrphidae sp.2	0	0	0	0	0	0	0	0	1	0	0	0	0
Syrphidae sp.3	0	0	0	0	0	0	0	0	0	0	0	0	1
Syrphidae sp.4	0	0	0	0	0	0	0	1	0	0	0	0	0
Syrphus sp.	0	0	1	0	0	0	0	0	0	0	0	0	0
Thyris sepulchralis	0	0	0	0	0	0	0	0	0	0	1	0	0
Toxomerus germinatus	0	0	0	0	0	0	1	0	0	0	0	0	0
Toxomerus marginatus	1	1	1	1	1	1	1	1	1	1	1	1	1
Villa fumicosta	0	0	0	0	0	0	0	0	0	1	0	0	0

Table S5. The table list means and standard errors of pollination services for the 13 *Arabidopsis lyrata* populations studied

Population	N° flowers recorded	Visitation rate [n°visits flower^{-1} day^{-1}]		Pollination rate [visited flow. obs.$^{-1}$ day^{-1}]		Pollinator richness [n°sp day^{-1}]		Shannon index		Simpson index	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
MD1	82	1.31	0.28	0.46	0.08	1.62	0.21	0.31	0.10	0.20	0.07
MD4	325	3.46	0.47	0.88	0.03	2.30	0.24	0.56	0.10	0.33	0.06
NC1	668	2.23	0.34	0.63	0.05	3.46	0.33	0.86	0.09	0.46	0.05
NC2	484	0.55	0.15	0.35	0.05	1.38	0.15	0.20	0.07	0.13	0.04
NC3	714	1.54	0.20	0.65	0.05	3.05	0.29	0.79	0.10	0.45	0.05
NC4	401	1.65	0.24	0.51	0.05	2.25	0.22	0.50	0.09	0.30	0.05
NY3	484	2.49	0.24	0.84	0.04	2.81	0.31	0.67	0.10	0.38	0.05
NY4	435	5.73	0.50	0.87	0.03	4.90	0.43	1.15	0.10	0.61	0.04
NY6	348	4.21	0.83	0.76	0.05	2.00	0.18	0.45	0.07	0.33	0.05
PA2	1338	2.30	0.23	0.80	0.03	3.50	0.21	0.88	0.06	0.49	0.03
VA1	1446	2.22	0.21	0.75	0.04	4.47	0.42	1.01	0.10	0.55	0.04
VA2	468	0.90	0.15	0.49	0.07	2.58	0.25	0.62	0.09	0.36	0.05
WV1	122	5.59	1.65	0.79	0.06	2.17	0.22	0.50	0.09	0.30	0.05

Table S6. **Population data on flowers and plant density for the 13 *Arabidopsis lyrata* populations studied**

Pop	Corolla width [mm]		Ovary lenght [mm]		Flower size [mm^2]		Local plant density [m^2]		Local flower density [m^2]	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
MD1	8.05	0.12	3.26	0.05	26.34	0.68	9.00	2.36	22.50	5.02
MD4	8.56	0.12	3.23	0.05	27.79	0.69	15.40	3.01	128.30	26.96
NC1	8.57	0.17	3.47	0.06	30.04	1.00	6.14	1.03	64.24	12.11
NC2	9.16	0.13	3.62	0.05	33.34	0.89	5.70	1.03	50.30	5.73
NC3	8.83	0.11	3.33	0.05	29.54	0.72	8.50	2.14	92.75	12.94
NC4	8.11	0.12	3.39	0.06	27.66	0.81	7.80	1.50	77.20	18.41
NY3	9.94	0.08	3.58	0.03	35.68	0.51	13.17	4.51	59.67	8.44
NY4	10.54	0.15	3.62	0.03	38.32	0.79	20.33	3.90	94.17	17.07
NY6	9.02	0.13	3.44	0.03	31.07	0.65	10.43	1.20	31.33	5.71
PA2	8.69	0.12	3.35	0.04	29.25	0.67	43.25	7.97	254.67	27.18
VA1	11.35	0.14	4.32	0.05	49.05	0.85	8.42	1.22	117.08	16.94
VA2	10.48	0.13	4.06	0.05	42.66	0.91	10.12	2.87	120.88	17.42
WV1	8.01	0.21	3.39	0.04	27.33	0.93	7.60	2.43	22.80	6.39

The table lists population means and standard errors of corolla width, ovary length and flower size based on 520 replicate flowers. Plant and flower density were extrapolated by precise counting on 12 1m^2 plots with *A. lyrata* occurrence.

Table S7. Population data on population census size and temperature conditions for the 13 *Arabidopsis lyrata* populations studied

Population	Population census size	Total flower census size	Plant species richness	Max T° [°C]	Mean T° [°C]	Min T° [°C]
MD1	0.6	1.4	0	23.7	21.7	19.0
MD4	91.2	760.2	2	25.7	22.4	17.9
NC1	1.1	11.8	5	24.0	19.3	12.8
NC2	28.7	253.1	1	29.5	26.4	23.0
NC3	5.8	63.3	3	22.1	17.3	11.9
NC4	9.0	88.7	5	36.2	29.2	18.2
NY3	135.1	612.4	2	23.8	18.9	12.1
NY4	65.5	303.4	5	21.8	16.9	11.0
NY6	74.9	225.1	7	26.9	23.5	16.8
PA2	378.1	2226.6	2	26.7	20.8	14.8
VA1	18.3	254.7	2	26.1	22.5	16.2
VA2	1.1	13.3	1	27.5	22.4	13.5
WV1	7.6	22.8	3	30.0	25.5	17.8

The table lists population plant and flower census size based on the density of 12 independent $1m^2$ plots and the total area where the population occurred. Both values are transformed x E03. Also, the maximum, mean, and minimum temperatures while cameras recorded.

Table S8. List of flowering plant species at each population

	Population												
Plant species	**MD1**	**MD4**	**NC1**	**NC2**	**NC3**	**NC4**	**NY3**	**NY4**	**NY6**	**PA2**	**VA1**	**VA2**	**WV1**
Alliaria petiolata	0	0	0	0	0	0	0	0	1	0	0	0	0
Amelanchier sp.	0	0	0	0	0	0	0	1	0	0	0	0	0
Antennaria plantaginifolia	0	0	1	0	0	0	0	1	0	0	0	0	0
Aquilegia canadiensis	0	0	0	0	1	0	1	1	1	0	0	0	0
Arabis sp.	0	0	0	0	0	0	0	0	0	0	0	0	1
Brassica sp.	0	0	0	0	1	0	0	0	0	0	0	0	0
Chionanthus virginicus	0	0	0	0	0	1	0	0	0	0	0	0	0
Erodium cicutarium	0	0	1	0	0	0	0	0	0	0	0	0	0
Geranium robertianum	0	0	0	0	0	0	0	0	1	0	0	0	0
Hesperis matronalis	0	0	0	0	0	0	0	0	1	1	0	0	0
Lamia sp	0	0	1	0	0	0	0	0	0	0	0	0	0
Lonicera sempervirens	0	0	0	0	0	1	0	0	0	0	0	0	0
Lonicera tatarica	0	0	0	0	0	0	0	0	1	1	0	0	0
Oxalis stricta	0	1	0	0	0	0	0	0	0	0	0	0	0
Phlox subulata	0	0	0	0	0	0	0	0	0	0	0	0	1
Potentilla norvegica	0	0	0	0	0	0	1	0	1	0	0	0	0
Ranunculus sp.	0	0	0	1	0	1	0	0	1	0	0	0	0
Saponnaria officinalis	0	0	0	0	0	0	0	0	0	0	0	0	1
Saxifraga paniculata	0	0	0	0	0	0	0	1	0	0	0	0	0
Senecio jacobaea	0	1	0	0	0	0	0	0	0	0	0	0	0
Silene sp.	0	0	0	0	0	0	0	0	0	0	1	1	0
Silene virginica	0	0	0	0	0	1	0	0	0	0	0	0	0
Vaccinium corymbosum	0	0	0	0	0	0	0	0	0	0	1	0	0
Vaccinium angustifolium	0	0	0	0	1	1	0	1	0	0	0	0	0
Veronica sp	0	0	1	0	0	0	0	0	0	0	0	0	0
Viola bicolor	0	0	1	0	0	0	0	0	0	0	0	0	0

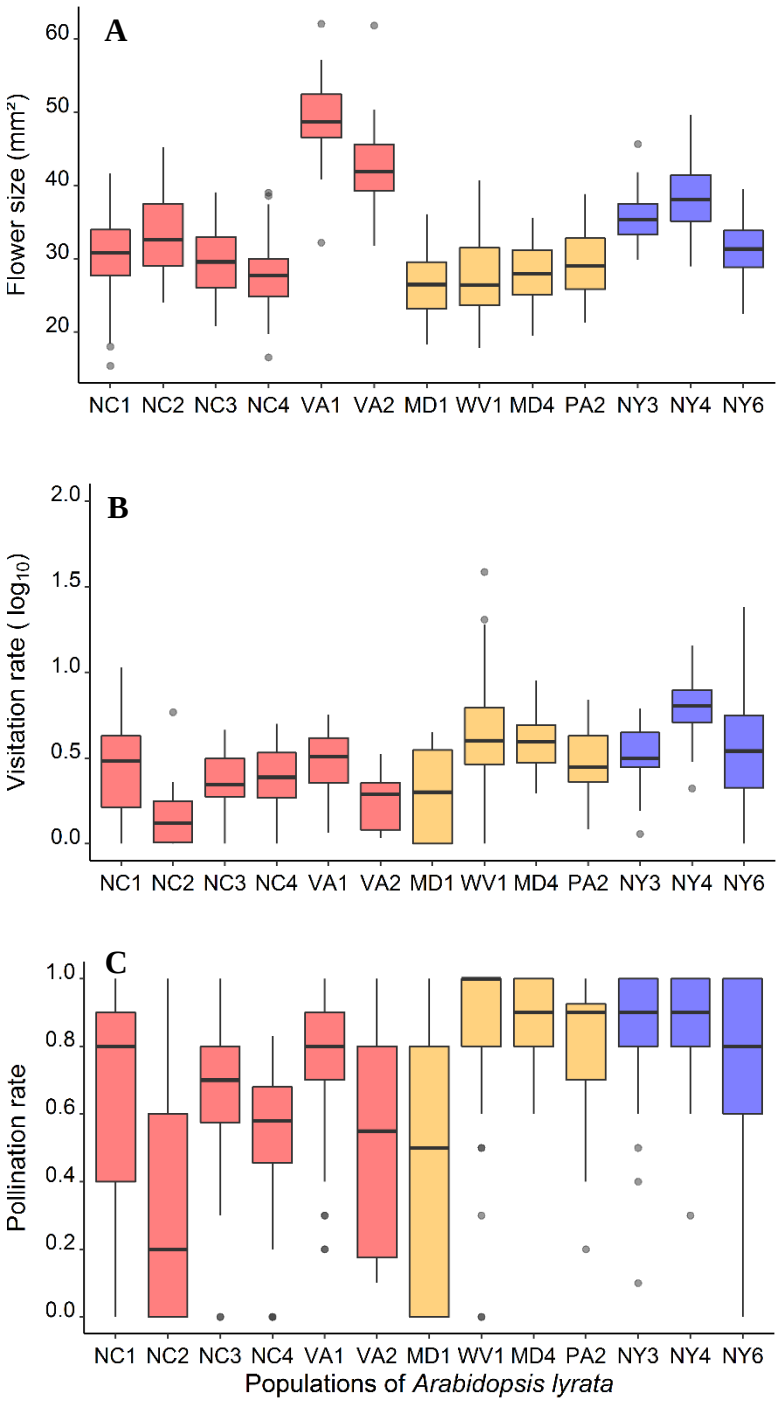

Figure S1. Flower size (A), visitation rate (B), and pollination rate (C) of each *Arabidopsis lyrata* population. The variation at each population corresponds to the several patches recorded and the daily replicates. Populations are sorted in the x-axis from most southern to most northern. The colour of the box plots indicates the position in the range of each population. Panel A shows box plots of flower size at each population. Panel B shows box plots of visitation rate at each population. Panel C shows box plots of pollination rate at each population. In red, orange, and blue are represented those populations coming from the south, centre and north respectively.

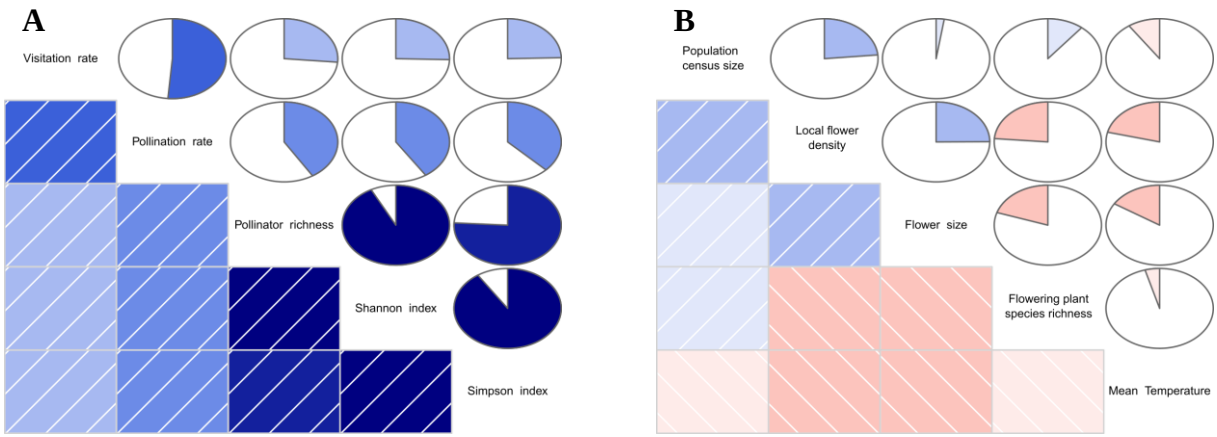

Figure S2. Matrix correlation between pollination services (A) and mechanisctic vairables (B). The color blue indicates a positive correlation between the variables, while pinkish indicates a negative correlation. The extent and intensity of the color in the pie chart indicates a greater correlation between the variables.

Chapter 4: **Temporal and spatial variation in a guild of pollinators in the North American *Arabidopsis lyrata***

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Abstract

Understanding the temporal and spatial variation of the plant-pollinator network is fundamental to predict future alterations and the consequences of speices lost. We studied insect pollinators visiting the North American *Arabidopsis lyrata* on several temporal and spatial scales. We recorded these interactions using time-lapse cameras over daytime, on replicate days, in two consecutive years, and at several sites within a total of 13 *A. lyrata* populations distributed over a latitudinal gradient of 1.100 km. We tested whether the temporal and spatial variation observed was related to temperature and the density of flowers respectively, and for a general signature of temporal niche partitioning among the different taxa. We found positive correlations between hourly visitation rate and temperature. Furthermore, the density of flowers explained partially the spatial variation, but the signature was taxon-specific. Most of the visits occurred during the central hours of the day indicating no evidence for niche partitioning, however, some insect groups had a wider spectrum in visitation along the day, becoming predominant for the pollination of the species at certain intervals. Overall, spatial and temporal variation in pollination services is a common phenomenon that occurs at different scales, which brings ecological flexibility for the organisms involved in the pollination network.

Introduction

The decline of insect pollinators in many parts of the world mostly because of changes in land use and human activity has been alarming the research community (Thomas *et al*. 2004; Biesmeijer *et al.* 2006; Hallmann *et al*. 2017). The decline of some functional groups of organisms can negatively affect other groups of species that rely upon them, known as community-level cascades, which can destabilize the entire ecosystem (Chapin *et al*. 1997). As most wild plant species and many agricultural cultivars are directly dependent on insect pollination for fruit and seed development (Ashman *et al*. 2004), understanding pollination networks has become a priority in ecology, and in agroecology in particular (Klein *et al*. 2007; Potts *et al.* 2010). However, pollination services are far from constant, varying in time and space due to extrinsic ecological conditions (Herrera 1990), but also due to intrinsic factors such as physiological tolerances and behavioral preferences (Gilbert 1985). This temporal and spatial variation in pollination services implies additional challenges to fully understand the mechanism and resilience of the interaction network in the community and to predict the consequences of species lost.

Most research on plant-pollinator interaction has considered temporal and spatial variation only to limited extents, as simultaneous records and complete daily observation periods imply tremendous efforts. But, plant-pollinator interactions do not occur continuously across time and space, instead, they have evolved to take place under optimum daily and seasonal rhythms and may differ depending on several aspects of the environment. The variation in pollination services has been shown at different scales, including: among plant species of the same habitat (Edwards *et al*. 2019), within the same plant species (Rader *et al*. 2012; Edwards *et al*. 2019), across years (Price *et al*. 2005), or even along with daily cycles (Knop *et al*. 2018). This variation can influence directly the ecological dynamics of the plant population density and communities that rely upon them. This has been shown experimentally,

where the decline of insect pollinators seemed to be paralleled with a reduction of plant reproductive success (Aguilar *et al*. 2006; Biesmeijer *et al.* 2006).

Groups of insects differ in their thermoregulatory optima and thermal tolerance (Paterson & Blouin-Demers 2017), and temperature is known to be one of the main environmental factors that influence the activity of the different pollinator taxa (Herrera 1990; Rader *et al*. 2012; Knop *et al*. 2017). Temperature-related activity patterns were shown to lead to common responses in pollination at the level of the species or larger taxonomic group (Willmer & Corbet 1981; Herrera 1990; Stone 1994; Vicens & Bosch 2000). For example, the activity rates of *Anthophora plumipes* in pollen and nectar transfer were related to air temperature depending also on body size (Stone 1994). Differences in flower attractiveness might explain also the spatial variation observed in pollination services. Plants tend to evolve characteristics that maximize the attraction of the pollinators and the effectiveness of the pollen transfer. This includes differences in plant height (Engel & Irwin 2003), the increase of flower display (Grindeland *et al*. 2005), the production of nectar, or the increase of flower size (Mitchell 1994). However, the characteristics and structure of the patches, such as flower display, is expected to affect pollination services more than floral traits as it offers a greater abundance of resources for the pollinators (Makino *et al*. 2007; Mayer *et al*. 2012). It seems clear that developing large floral displays with a greater amount of resources increases the possibility for the plant to be visited, i.e. plant visitation rate (reviewed by Ohashi & Yahara 1999). However, the total number of interactions that a flower receives, i.e. flower visitation rate, has been found to increase (Klinkhamer *et al*. 1989; Andersson 1991), to decrease (Andersson 1988; Klinkhamer & de Jong 1990; Grindeland *et al*. 2005) or even to be constant (Robertson & Macnair 1995; Vaughton & Ramsey 1998; Ohashi & Yahara 2002). Variation in plant-pollinator interactions on a short time scale may arise a temporal niche partitioning pattern. This occurs when some groups of the insect community select to forage during specific

times of the day, possibly in part to avoid resource competition (Stone *et al*. 1999). Partitioning might be an adaptation to facilitate the coexistence between organisms, which denotes ecological, evolutionary, and physiological implications (Kronfeld-Schor & Dayan 2003). Differences in activity windows are expected to explain part of daily partitioning and were suggested to be mainly determined by intrinsic factors such as physiological tolerances and behavioral responses to ecological dynamics (Stone 1994). The temporal variation in pollination could be a strategy that guarantees the reproduction of the plant species and resource capacity for pollinators. How the time of the day mediates ecological interactions, influences the structure of the community, and how evolution maintains such rhythms remains open (see review Kronfeld-Schor & Dayan 2003).

Despite the common concern on insect pollinators decays and the large volume of literature on plant-pollinators interactions, little is known about the patterns of change in most pollinator assemblages across time and space and the causes behind such variation. In the present study, we attempted to explain the variation across different scales including ecological factors, physiological tolerances, and behavioral preferences for each particular taxonomic group of pollinators. We recorded pollination services using time-lapse cameras on the plant species *Arabidopsis lyrata* subsp. *lyrata* over two years at 13 different populations along the 1.100km latitudinal gradient. We monitored simultaneously 10-12 separated patches with differences in the density of flowers at each population and assessed the variation in the pollination activity from 8:00 to 20:00. The large scale and detailed data of this study allowed us to address the following questions: Is the temporal variation in pollination services mainly explained by hourly temperature differences? (research question I). Is the spatial variation described by preferences in attractiveness related to the density of flowers? (research question II). Do taxonomic groups show different preferences toward temperature regimes and/or density of flowers? (research question III). Are pollinators more abundant at certain hours of

the day? (research question IV). Do pollinators show a signature of temporal niche partitioning? (research questions V). Do insect orders and Diptera taxa show differences in niche partitioning? (research question VI)

Material and Methods

Study organism

For the study of temporal, spatial variation, and temporal niche partitioning in pollination services, we focused on a particular plant species, the North American *Arabidopsis lyrata* subsp. *lyrata*. The plant species has a well-defined distribution from the south in North Carolina and Missouri to Upstate New York and south-western Ontario (Willi & Määttänen 2010, Willi *et al.* 2018), facing diverse ecological conditions along the gradient (Lee-Yaw *et al*. 2018). In total, thirteen populations were selected and monitored in a 1.100km latitudinal gradient, from North Carolina to upstate New York (Fig. 1, Table S1). The species can be found on sand dunes and rocky outcrops, sandy or rocky riverbanks, and rocky shorelines. In the Appalachians, plants grow on poor soils made of leaf litter and moss on the top of the raw rock but also on pure sand, under evergreen trees dominated by Virginia pine (*Pinus virginiana)* and the Eastern Red Cedar (*Juniperus virginiana)* that create a bit of shield. The plant is mostly a self-incompatible and insect-pollinated that produces a basal rosette with inflorescences emerging from the center during the blooming period (Fig. 1B). The number of flowers can vary from few to over 50 depending on the population and plant age, and they are known to produce nectar discs at the base of the anthers and volatile compounds to attract pollinators (Peer & Murphy 2003). A previous study on one population in Isle Royale found that the composition of the pollinators visiting *A. lyrata* flowers were mostly syrphids (Edwards *et al*. 2019).

Record of pollinators

Pollinator observations in the field might lack resolution due to the narrow sampling interval (Rafferty & Ives 2011, Hargreaves *et al*. 2015), due to the effect of the researcher on the insect behavior (Peckham & Peckham 1905), and due to the difficulties to identify live pollinators. Based on Edwards *et al*. (2015) research, we used time-lapse cameras (TLC 200 Pro HDR, Brinno, Taipei City, Taiwan) to monitor flower-insect interactions as an alternative to personal observations. The cameras make time-lapse photographs of the same vantage point over days and on simultaneous patches**,** allowing the identification and quantification of visitors on a diverse flower types (Edwards *et al*. 2015). The observation period started in mid-April, during peak flowering at the southern-most sites and extended to the beginning of June, when northern-most populations were in peak flowering for two consecutive years, in 2018 and 2019. Although repeated records would be ideal, we were not able to monitor all populations in both years. In each *A. lyrata* population, 10 to 12 flower patches per population were selected to be monitored simultaneously for three consecutive days for a 12-hour period, from 08:00 to 20:00 hrs (see Table S1 for detailed sample size). We did not conduct nocturnal observations as flowers close during the night. In summary, there are three different temporal scales: at the level of the hour, day, and year. Also, two different spatial scales: at the level of the patch within the populations, and between populations along the gradient. In this research, we focused on the hourly temporal scale, and on the patches within the population giving the following sample size: 13 populations x 1-2 years x 10-12 cameras x 2-4 days x 12 hours= 4.522 hours. We followed Edwards *et al*. (2015) protocol in setting the time-lapse cameras to shoot every 3 seconds, which has shown to capture 90% of total visits. Cameras were placed in the field for recording only under sunny and clear sky days.

After monitoring, videos were examined with *Quick Time Player* (Apple, California, US). The interaction was considered only if there was direct contact between the insect with

the pistil or stamens of the *A. lyrata* flower. The presence of ants was observed in all populations, however, they were discarded from the analysis due to their generally small size and their suggested ineffectiveness contribution as pollinators (Junker *et al.* 2007). Weevil beetles were observed in one population, but they were not considered in the analysis either due to their small size and complexity for quantification. In some populations, we found *Meligethes*, a Coleoptera that feeds on Brassicaceae flowers and therefore nor considered as pollinator. At the beginning of the video, only mature and fully open flowers were selected and assigned a specific ID. We calculated *hourly visitation rate* (n° visits flower^{-1} hour^{-1}) as the abundance of flower-insect interactions observed for a particular hour, divided by the total number of open flowers monitored.

Insect identification

Each flower-insect interaction was saved as an image for identification of the pollinator independently of their effectiveness, together with the ID of the flower visited, the day, the patch recorded, the time, and temperature. Insect identification was carried to the lowest taxonomic level given the quality of the images, using the identification keys by Kits *et al*. (2008), Miranda *et al*. (2013), Skevington *et al*. (2019). We first categorized pollinators based on the most frequent insect orders observed: Hymenoptera, Diptera, and Lepidoptera, or "unidentifiable" if the pollinator was not recognizable. Additionally, we classified Diptera visitors in the following taxa groups: Bombyliidae, Syrphidae, Muscoidea, and Empididae. Hymenoptera was split into morphotypes based on main phenotypic characters: morphological features, size, and color patterns. We then calculated hourly visitation rate for each taxonomic group.

Temporal and spatial characteristics

Hourly *temperature* and the local *density of flowers* were hypothesized as mechanisms that could potentially explain the variation of pollinators in time and space (research questions I, II, and III). The hourly air temperature was measured with two temperature data loggers (DS1922L, Maxim *iButton, CA, USA*) placed 1.5m above the ground and under the tree shadow. The *density of flowers* was estimated at each patch monitored as the total number of flowers in one m^2. *Temperature* was a variable estimated on the level of hour recorded in a population for each replicate day and year, while the *density of flowers* was a variable estimated on the level of a monitored patch of each population and year.

To understand whether pollinators had a greater abundance at certain intervals of the day and whether there was a signature of temporal niche partitioning (research questions III, IV, and V), we categorized the daily hours as *morning*, from 8 to 11:59; *mid-day* from 12:00 to 15:59; and *evening* from 16:00 to 19:59.

Statistical analysis

All main analyses were performed on hourly visitation rate of all *insecta* observed as a dependent variable. To test whether temporal variation was explained by hourly *temperature* (research question I) and whether the spatial variation was driven by the *density of flowers* in the patch (research question II), we performed a first analysis based on generalised linear mixed-effects model (GLMM). The analysis was performed in a Bayesian framework (MCMCglmm in R; Hadfield 2009; R Core Team 2019) because hourly visitation rate was 0-inflated and required the analysis of both the logistic part with the 0s and the Gaussian part of the distribution (values $\log_{10}$-transformed if >0). Fixed effects were hourly *temperature*, the *density of flowers* per m^2 ($\log_{10}$-transformed), and the interaction between both predictors. Random effects were daily replicate in a population and year, and population.

We also investigated whether the different taxonomic groups had specific preferences toward these predictors (research question III). The dependent variable here was the hourly visitation rate observed for each main insect order (Hymenoptera, Diptera, and Lepidoptera), and on the main Diptera taxa (Bombyliidae, Muscoidea, Syrphidae, Empididae) using GLMM and Bayesian statistics. Fixed effects were again the *density of flowers* per m^2 (log_{10}-transformed), hourly *temperature*, and the interaction between both predictors. Random effects were daily replicate in a population and year, and population.

In a secondary analysis, we tested whether there were differences in visitation rates across the daily interval (research question IV) and whether the different taxa showed a signature of niche partitioning (research questions V and VI). Fixed effect was the categorical interval of the day, *morning* or *evening* compared with *mid-day*. Random effects were daily replicate in a population and year, and population. All analyses were run on 5 parallel chains, with a burnin of 5000, thining of 150, and a nitt-value of 200,000.

Results

The total observation period included 4.522 hours of monitored flowers of natural *Arabidopsis lyrata* populations. Considering all patches, daily replicates, and populations, 7.310 flowers were examined, and 17.508 flower-insect interactions were observed (see Table S2 for a detailed summary of flowers recorded, insect abundance, visitation rate, the density of plants and flowers, and temperature conditions based on means per populations with SE). Of the total pollinators, we were able to identify 88% to the taxonomic order. About 48.9% of the pollinators were Hymenoptera, 47.7% Diptera, and only 3.2% Lepidoptera. The relative representation of the three insect orders varied among populations of *A. lyrata* (see Table S3). Most Diptera pollinators were of the families Syrphidae and Bombyliidae (46% and 32% respectively), followed by Muscoidea and Empididae (see Table S4). Bombyliids were

predominant at southern latitudes; while Syrphids were more frequent toward the north. In total, 67 different taxa were spotted to pollinate *A. lyrata* across the 1100 km gradient (listed in Table S5, and some images in Fig.S1). Some of the taxa were common in several locations, like the hoverfly *Toxomerus*, which was spotted as a common visitor in all populations recorded. Other pollinators were unique for each particular population and even patch recorded.

Is the temporal variation in pollination services mainly explained by hourly temperature differences? Is the spatial variation described by preferences in attractiveness related to the density of flowers? Do taxonomic groups show different preferences toward temperature regimes and/or density of flowers? (research questions I, II, and III).

As Figure 2 shows, temporal and spatial variation in pollination services is a common pattern that occurs in different scales; among population, between days and patches of the same population, and even between years. Differences in visitation rate between populations are expected as they occur in different habitats across the distribution range. Within the same population, the visitation rate and richness of pollinators also varied among the spatial replicates recorded simultaneously and between the daily replicates (Fig. 2 left panel). Differences were also observed for the two populations monitored in two consecutive years. The population in North Carolina increased the visitation rate and pollinator richness in 2019, while the population in upstate New York had similar values of visitation and richness as previous year (Fig. 2 right panel).

A first model tested for the effect of hourly *temperature*, the local *density of flowers*, and the interaction between both predictors on hourly visitation rate for *insecta* as a primary variable, i.e. all pollinators observed without differentiating due to taxonomy (Table 1). The effect of *temperature* was significant in the logistic part of the model, depicting the interaction

between flowers and insects, *temperature* was positively correlated with the occurrence of such event. For the log-normal part of the model, depicting the total number of interactions occurred per flower, *temperature* was also significantly correlated with hourly visitation rate. Figure 3 combines results of the two parts of the distribution, illustrating an increase of visitation rate as hourly temperature increases, however, there is an optimum that when is exceeded, the visitation rate starts decreasing. No significant effects were observed between the *density of flowers* and the hourly visitation rate neither for the logistic part or the log-normal part of the model. Moreover, no interaction was observed between *temperature* and the *density of flowers*. Deeper analyses focused on the insect orders have shown slightly similar patterns. Hourly visitation rates in the Hymenoptera and Diptera were both significantly correlated with *temperature* for both parts of the models. For Lepidoptera, only in the logistic part of the model's *temperature* was significantly correlated with hourly visitation rate. Additionally, for Hymenoptera there was a significant correlation between hourly visitation rate and the *density of flowers* for the log-normal part of the model, depicting the total number of interactions. But also, for the log-normal part of the model for Lepidoptera and density of flowers. This indicates that Hymenoptera and Lepidoptera are both attracted to patches with a higher density of flowers contributing to a greater number of interactions per flower. For Lepidoptera, there was a significant negative correlation between *temperature* and *density of flowers* on hourly visitation rate for the log-normal part of the model.

A deeper analysis of Diptera taxa is also presented in Table 1. For Bombyliidae, Empididae, and Syrphidae, a significant correlation was found with *temperature* in the logistic part of the distribution, and for Syrphids also in the log-normal part. Interestingly, Bombyliidae visitors showed a negative correlation with the *density of flowers* for the log-normal part of the model. This suggests that Bombyliids avoid patches with lots of flowers. However, a positive interaction was observed between the hourly visitation rate of Bombyliids with *temperature*

and *density of flowers*. For Muscoidea, no significant effects were observed neither for *temperature* or *density of flower*. Figure S2 shows the correlation of hourly visitation rate for *temperature* (x-axis) and *flower density* (y-axis) for Bombyliidae and Lepidoptera. The representation of the effect of density of flowers on visitation rate including all pollinators for each population is shown in Figure S3. There are some cases where the highest density of flowers seems to increase the visitation rate (see population MD1, NC4); others where the density of flowers show a decrease in visitation rate (see populations NC1, PA2, VA2) or even no change among the patch in terms of flower density (see populations NC3, VA1).

Are pollinators more abundant at certain hours of the day? Do pollinators show a signature of temporal niche partitioning? Do insect orders and Diptera taxa show differences in niche partitioning? (research questions IV, V, VI)

A second model tested for the effect of the hourly interval (*morning*, *mid-day*, *evening*) on visitation rate for *insecta* which included all pollinators observed (Table 2). The effect of daytime was significant in the logistic part and the log-normal part of the model for *morning* and *evening*. Values were significantly lower in the *morning* and evening compared to the *mid-day* interval. About 61% of total visits occurred during the *mid-day* hours (from 12:00 to 15:59); 9.6% of total visits occurred during *morning* (from 8:00 to 11:59); and, 29.2% happened in *evening* (from 16:00 to 20:00). As Figure 4 shows, when including all visitors, there is a well-defined bell shape in the abundance of pollinators, where the highest values coincided with the centre hours of the day. Inline, similar results were observed when pollinators were categorized in the three main orders (Table 2). Hourly visitation rates for Hymenoptera, Diptera, and Lepidoptera were significantly lower in the *morning* and late in the evening compared to *mid-day* hours. However, when looking at the four Diptera taxa, less pronounced differences were

found between the three intervals of the day (Table 2). While Bombyliidae, Empididae, Muscoidea, and Syrphidae all showed significant differences in the logistic part of the models; for the log-normal part of the model, only significant differences were found for Bombyliidae in the *morning* and Syrphidae in the evening. No differences were observed for Muscoidea or Empididae in the log-normal part for any of the intervals. As figure 4 shows, different distribution of the abundance are observed depending on the insect taxonomic groups at the order and Diptera taxa level. Some taxa had a broader peak in hourly activity, such as Syrphids, while others had a narrower peak in the hourly day, such as Bombyliids and Empidids. Some differences could be expected for the abundance of visitors at each particular population (see Figure S4), but in general, all populations followed a bell shape pattern where the highest abundance of insects occurred in the central hours of the day (but see MD4 where the highest peak of abundance occurred at 9:00).

Results indicated that there was a peak in visitation rate during the *mid-day* hours for almost all insect groups independently of their taxonomic category. But, some pollinators were more tolerant than others under certain time intervals. Figure 5 shows the density plot of insect orders abundance across daytime (panel A), or across temperature (panel B). Dipterans were the primary pollinator in the early *morning* as well as during the hottest temperatures. Lepidopterans were common during *mid-day* and late evening (Panel A) and have a higher tolerance of lower temperatures (Panel B). Analogous graphs for the Diptera taxa (Panel C and D), revealed that Syrphids were the main visitor in the early *morning*, were then partially replaced by Empididae and Muscoidea, and finally, Bombyliidae dominated in the mid-afternoon. The Muscoidea were found to be the most tolerant under the warmest temperatures. Similar density plots for each *A. lyrata* population are shown in Figure S5 (referring to the main insect orders) and Figure S6 (for the Diptera taxa).

Discussion

Remarkable aspects of the present study rely on the large sample size including the number of populations, the simultaneous spatial records within the population, the several temporal samples across days, and along the daily hours. Our results confirm that pollination services are far from constant as they vary across the time of day, with temperature and to a lesser extent with flower availability. Furthermore, taxonomic groups of pollinators differ in their response to the latter two variables, but particularly to temperature.

For the North American plant species *Arabidopsis lyrata* we found that the most common pollinators were wild bees, Syrphids, and Bombyliids. However, the contribution of each group varied depending on the population. We have seen that some taxa were present in all sites, like the hoverfly genus *Toxomerus*, however, some pollinators were observed rarely or unique for a particular habitat. Our results show that the plant-pollinator network is a generalist system, which provides ecological flexibility in terms of reproduction for the plant species, but also a diverse of food resources for the pollinators. The importance of pollinator richness not only guarantees plant reproduction in case one of the pollinator species go extinct but also contributes to enhancing the fruit and seed set. This has been shown in a manipulated experiment on main pollinators groups of *Raphanus sativus* where the richness of the pollinators increased significantly the fruit and seed production (Albrecht *et al*. 2012). However, not all flower visitors act as pollinators (Wackers *et al.* 2007); those who do also vary in their effectiveness in pollinating and their frequency depending on the insect group (Ne'eman *et al.* 2010). The different morphological and behavioral characteristics distinctive of each insect group might contribute differently to the success to pollinate. For example, the contribution of bees actively searching and rubbing floral organs is probably more efficient than the gentle landing of Syrphids on the flowers. Even though bees might be one of the most

efficient and abundant pollinators for the ecosystem services, it has also been shown that other insects can contribute substantially to the pollination services with similar values of pollen deposition and frequency of visits as bees (see Rader *et al*. 2012).

Temperature and temporal variation in visitation rate:

From figure 3, we see there is a polynomial distribution in the visitation rate of pollinators with hourly temperature conditions. Our study has shown that the effect of hourly temperature was positively correlated with visitation rate of the pollinators independently of the taxon group. However, we have observed some taxa were more tolerant than others for a range of temperature experienced. For example, Lepidoptera was one of the main pollinators under colder temperatures, while Muscoidea dominated the spectrum at higher temperatures. This indicates that temperature is a primary ecological driver that explains the temporal variation along the daily hours. This is in accordance with previous studies that have shown similar results (McCall & Primack, 1992; Herrera 1990; Rader *et al.*2012; Knop *et al.* 2018). However, it has been shown that other ecological factors such as brightness (Knop *et al*. 2017; Knop *et al*. 2018) or precipitation of previous years (Moeller *et al*. 2012) also affect pollinator activity. However, it is known that the variation of these ecological factors not only affects the pollination activity, but also the physiology of the flower attractiveness, modifying for example the production of nectar (Kenoyer 1917; Huber 1956).

Spatial variation and the effects of local flower density in visitation rates:

We can not confirm that the differences in the density of flowers could explain the variation in visitation rates considering all pollinators as a whole. While some studies reported a positive correlation between the density of flowers and visitation rate (Kunin 1993; Delmas *et al.* 2016; Edwards *et al*. 2019), others found no effect of flower density on visitation rate in the biennial *Echium vulgare* (Klinkhamer & de Jong 1990), or in the perennial *Cirsium purpuratum* (Ohashi

& Yahara 2002), and even a negative correlation was found for the herbaceous biennial *Digitalis purpurea* (Grindeland *et al*. 2005) or in the annual herb *Lasthenia californica* (Hendrickson *et al*. 2018). However, there is no reason to think that all insect taxa should have similar preferences toward flower attractiveness. Our results have shown that it might be taxon-dependent. Hymenoptera and Lepidoptera were attracted to high dense patches, which increased the visitation rate per flower; other groups did not seem to be affected by the density of flowers such as Syrphidae or Muscoidea; while for Bombyliidae pollinators, in contrast, seemed to evade big flower density patches. This was previously shown by Sih & Baltus (1987), as the relationship between the density of flowers and flower visitation rate in the perennial *Nepeta cataria* depends on the pollinator taxa. They found honey and bumble-bees shown a preference toward high dense patches, while negative for solitary bees. The implications that the variation in flower density alter the pollinator behavior at the level of the population might have consequences in pollen flow and the limits of the populations. If pollinators prefer high dense patches, the core of the population might receive a greater abundance of visitors than the edges where the density of plants and flowers are expected to decrease. For this reason, some pollinators might be particularly important such as Bombyliids in the leading edge for the contribution of the reproduction and expansion/retraction dynamics. Additionally, sites that do not share the same pollinators taxa, gene flow may remain local even in small scales in the population (i.e. if two flowers do not share the same pollinators species, we can assume there is no gene exchange between them). This could imply gene flow limitation in patches that are not physically separated by a barrier but separated by different pollinator groups.

Other aspects related to attractiveness and pollinator preferences as the position of the flower in the plant have been suggested to affect the visitation rate. For example, Albrecht *et al*. (2012) demonstrated that high flowers received more visits than flowers in lower inflorescences. The differences in insect taxa and visitation rates among patches might be also

explained by differences in microclimate and proximity of resources. Edwards *et al*. (2019) proposed that insects forage in the local area, on flowers within their home range. The experiment took place in Isle Royale on ten plant species where differences in pollinator taxa and abundance were found between two sites separated approx. 400m.

Temporal niche and resource partitioning,

The abundance of insect pollinators across the day followed a perfect bell shape, where the peak coincided with the central hours of the day. This supports similar results found by Albrecht *et al*. (2012) and Knop *et al.* (2018) where the highest numbers of visits occurred also during mid-day and decreased in the morning and the evening. However, the complete interaction network can be underestimated when only focusing on a partial interval of the daytime. Knop *et al.* (2018) have shown throughout 24-h observations that even though the abundance and species richness was greater during the mid-day hours, specific taxa visited only during the night. Albrecht *et al.* (2012) showed on three major functional pollinator groups that the differences in the diurnal activity patterns were the main mechanism that facilitated the coexistence of pollination success in *Raphanus sativus*. In our study even though the major abundance of insects was observed during the central hours of the day (Fig. 4), some groups were more tolerant and become predominant pollinators under certain hours. For example, Syrphids were the main visitor in the mornings while Muscoidea for late in the evening (Fig. 5). In the case of Syrphids, different dial periodicities have been shown from early in the morning to late in the evening depending on the species, and/or their activity (Gilbert 1985). We did not find signatures of niche partitioning between the different insect groups across the three categorical intervals as most of the pollinators decreased from *mid-day* to *morning* or *evening* intervals. Albrecht *et al.* (2012) did not find either significant differences in visitation rates among the daytime divided into four periods for the main insect groups. However, they found trends were

solitary bees and syrphids tended to visit early in the morning and noon, while social bees visited most of the flowers between 14 and 16:30. Niche temporal partitioning is supposed to enhance the interaction network as provides a diverse community of visitors (Hoehn *et al.* 2008; Fontaine *et al*. 2006; Blüthgen & Klein 2011). Taking advantage of the different spatio-temporal niches, plants can benefit from greater reproduction success while maximizing the available resources for the visitors. However, the partitioning of the resources might be underpinned at a finer scale, (i.e. at a lower taxonomic level), or habitat-specific (i.e. the interaction network in northern populations might have different dynamics than populations in southern latitudes).

Conclusion

The pollination network is far from being a stable system across time and space, which implies ecological flexibility ensuring the persistence of the species involved (Waser *et al*. 1996). Here we found that the diurnal pollinator activity is mostly driven by temperature regimes. Overall, the abundance of pollinators was greater during the central hours of the day. However, some pollinators seemed to be more tolerant to lower or higher temperature regimes and hourly intervals than others, enhancing the spectrum of the temporal niche. Spatial variation can be partially explained for certain groups by inner behavioral preferences toward high-density flower patches, however other ecological variables and physiological responses are involved in the pollinator activity that could also help to better understand part of the variation.

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Table 1. Summary of model testing for the effect of temperature, density of flowers, and their interaction on hourly visitation rate of all insects, the three main insect orders, and for the main four Diptera taxa observed *Arabidopsis lyrata* flowers

		Fixed effects, logistic process								
		Temperature			**Density of flowers**			**Temperature * density of flowers**		
	N	**Mean**	**HPD**		**Mean**	**HPD**		**Mean**	**HPD**	
Depend. variable										
All pollinators										
Insecta	1300	**0.184**	**(0.145,0.230)**	***	0.159	(-0.341,0.771)		-0.002	(-0.027,0.017)	
Main orders										
Hymenoptera	1300	**0.213**	**(0.156,0.272)**	***	**0.900**	**(0.091,1.587)**	*	-0.023	(-0.053,0.006)	
Diptera	1300	**0.106**	**(0.054,0.157)**	***	-0.098	(-0.764,0.527)		0.005	(-0.020,0.033)	
Lepidoptera	1300	0.102	(-0.030,0.229)		0.679	(-1.013,2.154)		-0.003	(-0.068,0.059)	
Diptera taxa										
Bombyliidae	1300	**0.126**	**(0.017,0.254)**	*	-0.021	(-1.423,1.285)		0.007	(-0.051,0.063)	
Empididae	1300	**0.299**	**(0.067,0.577)**	**	0.961	(-2.121,4.064)		-0.079	(-0.219,0.055)	
Muscoidea	1300	0.030	(-0.066,0.139)		-0.563	(-2.004,0.945)		0.029	(-0.022,0.084)	
Syrphidae	1300	**0.097**	**(0.038,0.151)**	***	0.272	(-0.436,1.047)		-0.010	(-0.041,0.020)	

		Fixed effects, log-normal process								
		Temperature			**Density of flowers**			**Temperature * density of flowers**		
	N	**Mean**	**HPD**		**Mean**	**HPD**		**Mean**	**HPD**	
Depend. variable										
All pollinators										
Insecta	1300	**0.038**	**(0.029,0.046)**	***	0.004	(-0.108,0.109)		-0.001	(-0.005,0.003)	
Main orders										
Hymenoptera	1300	**0.045**	**(0.031,0.059)**	***	0.149	(-0.028,0.302)		-0.005	(-0.011,0.002)	
Diptera	1300	**0.019**	**(0.010,0.031)**	**	-0.084	(-0.218,0.043)		0.001	(-0.004,0.007)	
Lepidoptera	1300	**0.044**	**(0.023,0.065)**	***	**0.393**	**(0.143,0.684)**	**	**-0.023**	**(-0.034,-0.011)**	***
Diptera taxa										
Bombyliidae	1300	-0.012	(-0.030,0.011)		**-0.267**	**(-0.425,-0.024)**	**	**0.008**	**(0.000,0.015)**	*
Empididae	1300	-0.019	(-0.123,0.091)		-0.407	(-1.797,0.889)		0.009	(-0.048,0.066)	
Muscoidea	1300	-0.003	(-0.029,0.024)		-0.061	(-0.443,0.326)		0.000	(-0.013,0.014)	
Syrphidae	1300	**0.021**	**(0.006,0.035)**	*	0.038	(-0.120,0.195)		-0.004	(-0.009,0.003)	

Hourly visitation rate (log_{10}-transformed if >0) followed a Gaussian distribution with 0-inflation. Therefore, models assessed all fixed and random effects for their importance in both the logistic process (binary variable depicting a visit occurred or not) and the Gaussian process (total rate of flower-insect interactions occurred). Estimates of coefficients are modes of an MCMC sample from the posterior distribution of parameters (*mean* and higher posterior density, *HPD*, interval). Estimates with *P*-values < 0.05 are written in bold; significance is indicated: (*) *P*<0.1, * *P*<0.05, ** *P*<0.01, *** *P*<0.001. Results for random effects are not shown.

Table 2. Results of model testing for the effect of time of day on hourly visitation rate of all insects, the three main insect orders, and for the main four Diptera taxa observed visiting *Arabidopsis lyrata* flowers

		Fixed effects, logistic process					
		Morning			Evening		
	N	Mean	HPD		Mean	HPD	
Depend. variable							
All pollinators							
Insecta	1300	**-1.255**	**(-1.351,-1.164)**	***	**-0.997**	**(-1.089,-0.915)**	***
Main orders							
Hymenoptera	1300	**-1.196**	**(-1.306,-1.071)**	***	**-1.180**	**(-1.300,-1.068)**	***
Diptera	1300	**-0.877**	**(-1.003,-0.762)**	***	**-0.554**	**(-0.655,-0.459)**	***
Lepidoptera	1300	**-1.020**	**(-1.273,-0.749)**	***	**-0.909**	**(-1.184,-0.626)**	***
Diptera taxa							
Bombyliidae	1300	**-1.134**	**(-1.376,-0.928)**	***	**-0.376**	**(-0.537,-0.212)**	***
Empididae	1300	**-4.309**	**(-9.230,-1.197)**	***	**-0.813**	**(-1.205,-0.428)**	***
Muscoidea	1300	**-0.928**	**(-1.262,-0.613)**	***	**-0.289**	**(-0.528,-0.070)**	*
Syrphidae	1300	**-0.584**	**(-0.718,-0.455)**	***	**-0.553**	**(-0.660,-0.447)**	***
		Fixed effects, log-normal process					
		Morning			Evening		
	N	Mean	HPD		Mean	HPD	
Depend. variable							
All pollinators							
Insecta	1300	**-0.256**	**(-0.278,-0.234)**	***	**-0.207**	**(-0.225,-0.187)**	***
Main orders							
Hymenoptera	1300	**-0.230**	**(-0.268,-0.195)**	***	**-0.232**	**(-0.273,-0.195)**	***
Diptera	1300	**-0.184**	**(-0.214,-0.147)**	***	**-0.121**	**(-0.144,-0.100)**	***
Lepidoptera	1300	**-0.112**	**(-0.164,-0.034)**	*	**-0.111**	**(-0.165,-0.034)**	**
Diptera taxa							
Bombyliidae	1300	**-0.118**	**(-0.171,-0.028)**	*	-0.041	(-0.073,0.006)	
Empididae	1300	-	-		-0.124	(-0.352,0.095)	
Muscoidea	1300	0.054	(-0.021,0.134)		-0.002	(-0.041,0.036)	
Syrphidae	1300	-0.090	(-0.131,0.014)		**-0.100**	**(-0.135,-0.019)**	*

The effect of the time interval is compared with the one in the mid-day (between 12:00-16:00). Hourly visitation rate (log_{10}-transformed if >0) followed a Gaussian distribution with 0-inflation. Therefore, models assessed all fixed and random effects for their importance in both the logistic process (binary variable depicting a visit occurred or not) and the Gaussian process (total rate of flower-insect interactions occurred). Estimates of coefficients are modes of an MCMC sample from the posterior distribution of parameters (*mean* and higher posterior density, *HPD,* interval). Estimates with *P*-values < 0.05 are written in bold; significance is indicated: (*) $P<0.1$, * $P<0.05$, ** $P<0.01$, *** $P<0.001$. Results for random effects are not shown.

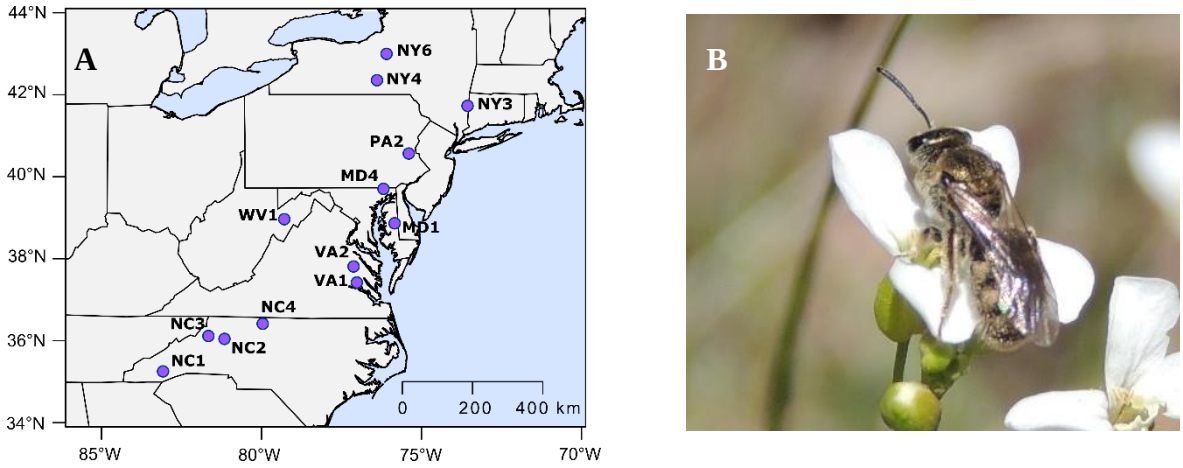

Figure 1. Distribution of the 13 selected *Arabidopsis lyrata* populations studied for the variation in pollination service in North America (A) and an image of *A. lyrata* flowers with a wild bee visiting (B). The map shows the populations indicated by dots accompanied by a three-digit abbreviation represent the sites of origin of populations (Table S1, the two letters stand for the state in the US, the number for latitudinal position within the state as in Willi *et al.* 2018).

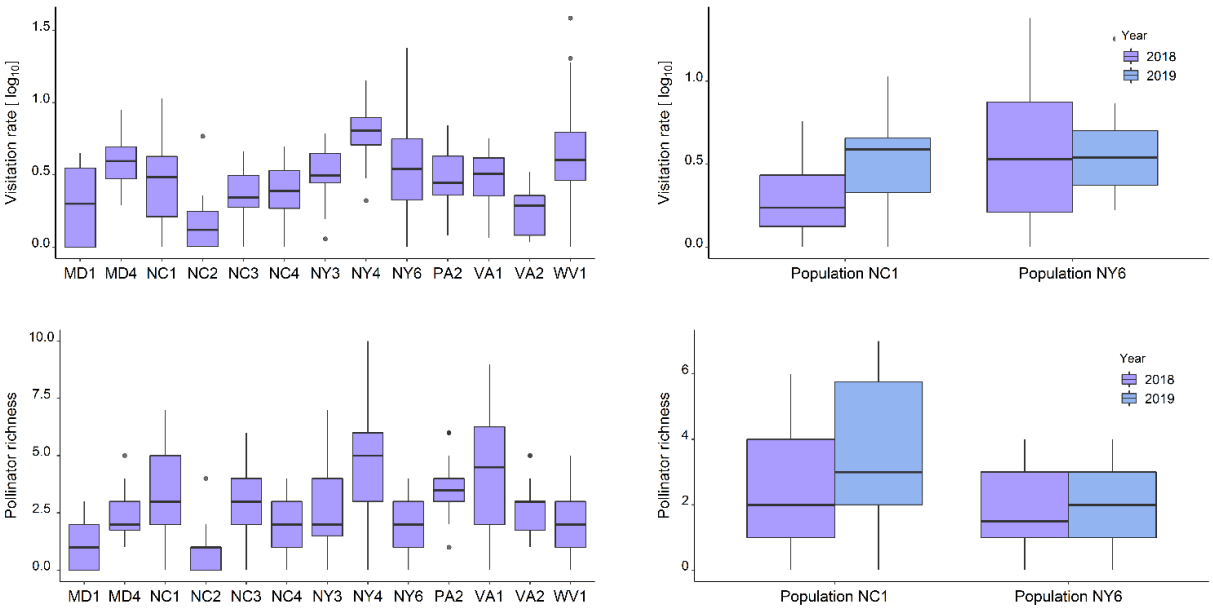

Figure. 2. Variation in visitation rate (top) and richness (bottom) of each *Arabidopsis lyrata* population recorded (left) and for the two populations recorded both years (right). The variation at each population corresponds to the several patches recorded and the daily replicates. Populations are sorted in the x-axis from most southern to most northern.

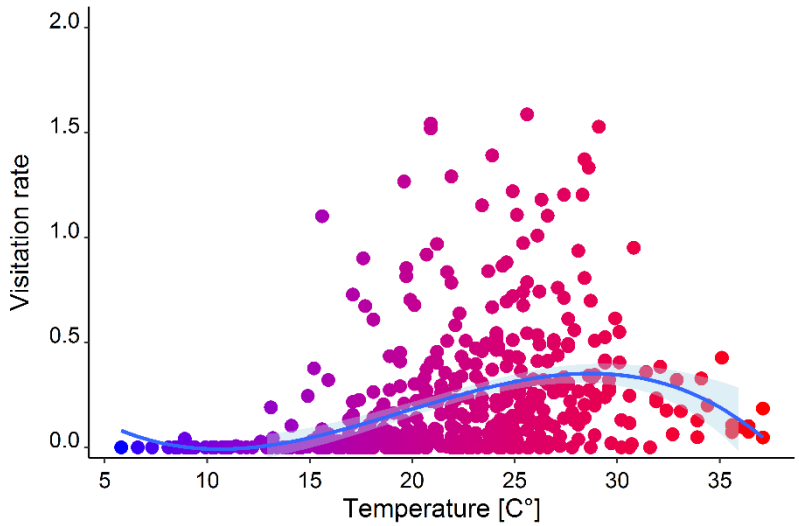

Figure 3. Relationship between hourly visitation rate and temperature on flowers of *Arabidopsis lyrata*. The scatterplot represents the predicted polynomial relationship between visitation rate and temperature in °C. Dots are hourly means based on patch means, for each population. The model-predicted regression line is shown in blue, with the lower and upper 95% confidence interval.

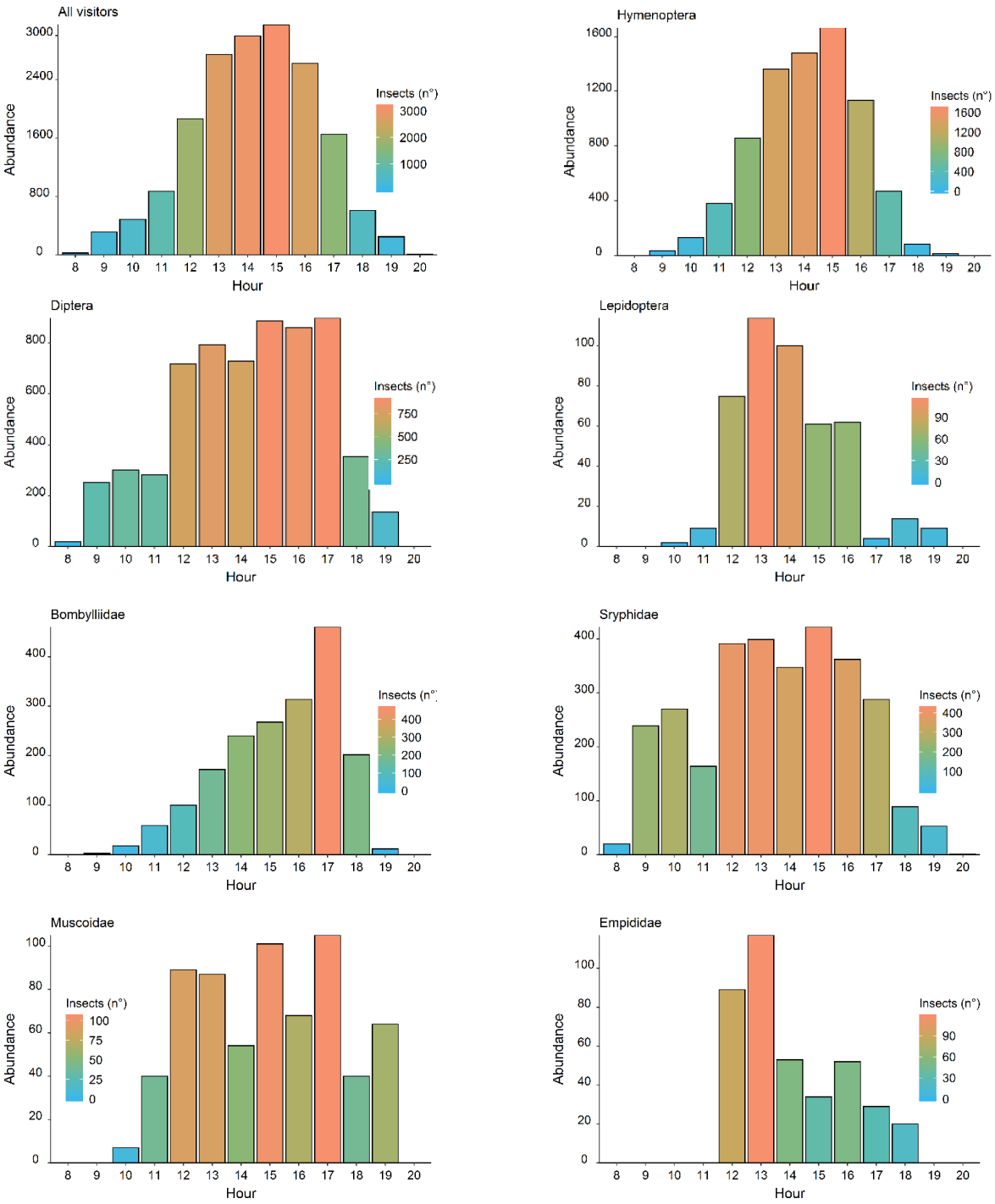

Figure 4. Relationship between pollinator abundance of all visitors, main insect orders, and Diptera taxa with hour of a day on *Arabidopsis lyrata* flowers. Each graph represents the abundance of insects observed plotted on the hour of the day including all populations, patches, and days monitored. The top-left graph shows the abundance including all visitors observed, while the rest of the graphs show the abundance of insect orders (Hymenoptera, Diptera, Lepidoptera) and the abundance of each Diptera taxa (Bombyliidae, Syrphidae, Muscoidea, Empididae). The greatest number of flower-insect interactions occurred during the central hours of the day.

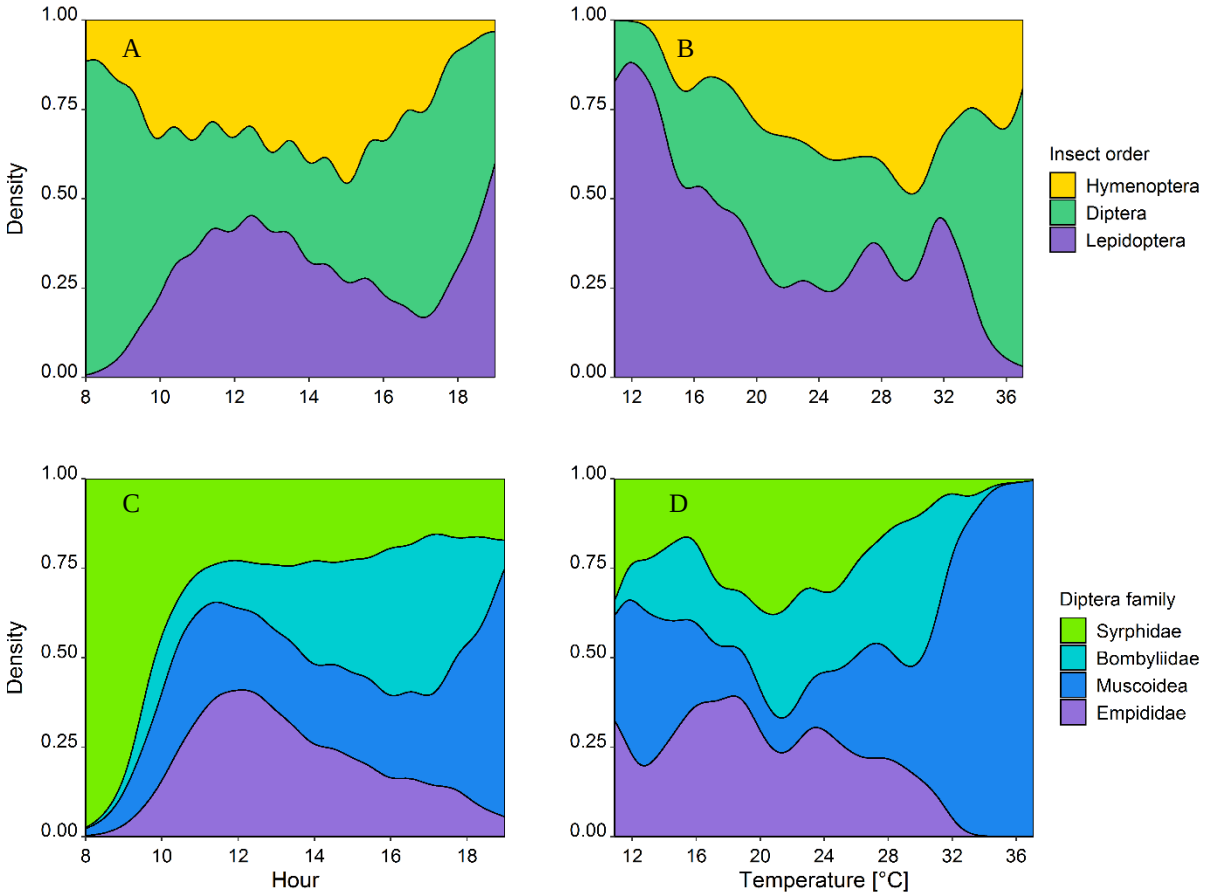

Figure 5. Density plot of insect orders (A and B) and Diptera taxa (C and D) visiting across the time of day (A and C), and temperature (B and D) for the 13 *Arabidopsis lyrata* populations monitored. Panel A shows the density of the main insect orders observed across time from 8:00 to 19:59. Panel B shows the analogous density plot on temperature. Panel C depicts the density of Diptera taxa across hours, while panel D shows also the density of Diptera taxa but across temperature regimes.

Supplementary information

Table S1. General information on the *Arabidopsis lyrata* populations studied

Pop	Location	Latitude [° N]	Longitude [° W]	Elevation [m a.s.l.]	Area [m^2]	Days of recording	N° of patches	Total hours
MD1	Martinak St. Park	38.86	75.84	2	64	2	10	208
MD4	Conowingo	39.70	76.19	79	5,925	2	10	262
NC1	Tuckasegee	35.25	83.08	1,015	184	3.5	21	379
NC2	Moravian Falls	36.04	81.16	680	5,031	4	20	331
NC3	Blowing Rock	36.11	81.66	1,108	682	5.5	6	400
NC4	Mayodan	36.41	79.96	224	1,149	3	9	308
NY3	Dover Plains	41.73	73.56	143	10,264	3	12	397
NY4	Ithaca	42.35	76.39	442	3,222	3	12	424
NY6	Clark State Park	43.00	76.09	229	7,185	4	20	418
PA2	Allentown	40.57	75.40	125	8,743	3	12	400
VA1	Sandybottom	37.42	77.02	5	2,175	3	12	428
VA2	Aylett	37.81	77.12	10	110	3	8	260
WV1	Hopeville	38.96	79.29	395	1,001	3.5	10	307

The table provides information for each of the populations studied: population abbreviation, location, latitude, longitude, elevation, surface area with occurrence; the number of days of recording pollinators; the number of patches in the population; and the total number of hours of recording.

Table S2. The table list means and standard errors of pollination services and field conditions for the 13 *Arabidopsis lyrata* populations studied

Pop	Flowers recorded	Abundance	Visitation rate [vis. flower^{-1} day^{-1}]		Plant density [m^2]		Flower density [m^2]		Mean T. [°C]		Max T. [°C]	Min T. [°C]
			Mean	SE	Mean	SE	Mean	SE	Mean	SE		
MD1	82	128	1.31	±0.28	9.00	2.36	22.50	5.02	22.9	0.7	23.7	19.0
MD4	325	1143	3.46	±0.47	15.40	3.01	128.30	26.96	23.3	0.6	25.7	17.9
NC1	668	1547	2.23	±0.34	6.14	1.03	64.24	12.11	19.3	0.6	24.0	12.8
NC2	484	198	0.55	±0.15	5.70	1.03	50.30	5.73	26.8	0.7	29.5	23.0
NC3	714	1179	1.54	±0.20	8.50	2.14	92.75	12.94	17.3	0.6	22.1	11.9
NC4	401	855	1.65	±0.24	7.80	1.50	77.20	18.41	29.2	1.0	36.2	18.2
NY3	484	1230	2.49	±0.24	13.17	4.51	59.67	8.44	18.9	0.5	23.8	12.1
NY4	435	2563	5.73	±0.50	20.33	3.90	94.17	17.07	17.1	0.7	21.8	11.0
NY6	348	1136	4.21	±0.83	10.43	1.20	31.33	5.71	23.5	0.5	26.9	16.8
PA2	1338	2769	2.30	±0.23	43.25	7.97	254.67	27.18	20.8	0.7	26.7	14.8
VA1	1446	3826	2.22	±0.21	8.42	1.22	117.08	16.94	22.8	0.8	26.1	16.2
VA2	468	385	0.90	±0.15	10.12	2.87	120.88	17.42	22.5	0.9	27.5	13.5
WV1	122	549	5.59	±1.65	7.60	2.43	22.80	6.39	25.5	0.5	30.0	17.8

The table provides information on sample size, means, and standard errors for each of the populations studied based on means per day and patch recorded: flowers recorded, abundance of insects observed, visitation rate, the plant density per one m^2, the flower density per one m^2, mean temperature based on hourly records with maximum and minimum.

Table S3. Order diversity rate of the main insect groups at each population of *Arabidopsis lyrata*

	Fraction rate of the main insect orders			
Population	**Hymenoptera**	**Diptera**	**Lepidoptera**	**Coleoptera**
MD1	42.1	57.9	0.0	0.0
MD4	22.6	77.0	0.2	0.1
NC1	41.6	43.9	14.4	0.2
NC2	57.7	38.6	3.7	0.1
NC3	57.4	42.0	0.0	0.7
NC4	32.1	67.7	0.0	0.0
NY3	19.2	74.7	5.9	0.3
NY4	51.9	48.0	0.0	0.1
NY6	69.8	29.8	0.2	0.2
PA2	47.3	50.8	1.8	0.1
VA1	70.8	26.6	2.6	0.0
VA2	61.1	32.4	6.6	0.0
WV1	62.7	31.1	6.2	0.0
Average	48.9	47.7	3.2	0.1

Table S4. **Diversity rate of the main Diptera taxa at each population *Arabidopsis lyrata***

Fraction rate of the main Diptera taxa				
Population	**Syrphidae**	**Bombyliidae**	**Muscoidea**	**Empididae**
MD1	62.9	0.0	37.1	0.0
MD4	98.3	0.1	1.6	0.0
NC1	69.6	26.8	3.6	0.0
NC2	13.9	86.1	0.0	0.0
NC3	29.3	69.6	1.1	0.0
NC4	12.7	0.7	86.7	0.0
NY3	90.1	7.9	0.0	1.9
NY4	26.6	11.3	16.2	46.0
NY6	86.0	1.5	12.5	0.0
PA2	46.0	33.9	20.1	0.0
VA1	9.1	87.2	3.0	0.7
VA2	6.5	91.9	0.0	1.6
WV1	47.2	4.3	5.0	43.5
Average	46.0	32.4	14.4	7.2

Table S5. List of species/morphotypes observed pollinating *Arabidopsis lyrata* sub. *lyrata* in North America

	Population												
Specie/ Morphotype	**MD1**	**MD4**	**NC1**	**NC2**	**NC3**	**NC4**	**NY3**	**NY4**	**NY6**	**PA2**	**VA1**	**VA2**	**WV1**
Alipia octomaculata	0	0	0	0	0	0	0	0	0	1	0	0	0
Anthocharis midea	0	0	1	1	0	0	0	0	0	0	0	0	0
Apocrita morphotype 1	0	0	0	0	1	1	0	1	1	0	0	0	1
Apocrita morphotype 2	1	1	0	1	1	0	0	1	0	0	1	0	0
Apocrita morphotype 3	0	0	1	0	0	0	0	0	0	1	0	0	0
Apocrita morphotype 4	0	1	0	0	0	0	0	1	0	1	0	0	0
Apocrita morphotype 5	0	1	0	1	0	0	0	0	0	0	0	0	0
Apocrita morphotype 6	0	0	0	0	0	0	0	0	0	0	1	1	1
Apocrita morphotype 7	0	0	0	0	0	0	0	0	0	0	1	1	0
Apocrita morphotype 8	0	0	0	0	0	0	0	0	0	1	1	0	0
Apocrita morphotype 9	0	0	1	0	0	0	0	0	0	0	0	0	0
Apocrita morphotype 10	0	0	0	0	0	0	1	0	0	0	0	0	0
Apocrita morphotype 11	0	0	0	0	0	0	0	0	0	0	0	1	0
Apocrita morphotype 12	0	0	0	0	0	0	0	1	0	0	0	0	0
Apocrita morphotype 13	0	0	0	0	0	0	0	0	0	0	0	0	1
Apocrita morphotype 14	0	0	0	0	1	0	0	0	0	0	0	0	0
Apocrita morphotype 15	0	0	1	0	0	0	0	0	0	0	0	0	0
Asterocampa sp.	0	0	1	1	0	0	0	0	0	0	1	1	1
Bombus sp.	0	0	0	0	1	0	0	0	0	0	1	0	0
Bombylius major	0	1	1	1	1	1	1	1	1	1	1	1	0
Bombylius pulchellus	0	0	0	0	0	0	0	0	0	1	0	0	0
Bombylius pygmaeus	0	0	0	0	0	0	0	1	0	0	0	0	0
Callophrys grynaeus	0	0	0	0	0	0	1	0	0	1	0	0	0
Chloropidae	0	0	0	0	0	0	0	0	1	0	0	0	0
Chrysotoxum sp.	0	0	1	0	0	0	0	0	0	0	0	0	0
Coleoptera	0	0	0	0	0	0	0	0	1	0	0	0	0
Conopidae	0	0	0	0	0	0	0	1	0	0	0	0	0
Cupido comyntas	0	0	0	0	0	0	0	0	0	0	1	1	0
Empididae	0	0	0	0	0	0	0	1	0	0	0	0	0
Empis sp.1	0	0	0	0	0	0	1	1	0	0	0	0	0
Empis sp.2	0	0	0	0	0	0	0	0	0	0	0	0	1
Empis sp.3	0	0	0	0	1	0	0	0	0	0	0	1	0
Epalpus sp.1	0	0	0	0	0	0	0	1	0	0	0	0	0
Epalpus sp.2	1	0	0	0	0	0	0	0	0	0	0	0	0
Eristalis saxorum	0	0	0	0	0	0	0	0	0	1	1	0	0
Eupeodes sp.	0	0	0	0	0	0	0	0	0	0	0	0	1
Eurythmia sp.	0	0	0	0	0	0	0	0	0	0	0	0	1
Halictidae	0	1	1	1	1	1	0	1	1	0	1	1	0
Heliophilus fasciatus	0	0	0	0	0	0	1	0	0	0	0	0	0
Hemipenthes sp.	0	0	0	0	0	0	0	0	0	0	0	0	1

Heteroptera	0	0	0	0	0	0	0	0	0	0	1	0	0
Mallota bautias	0	0	0	0	0	0	0	0	0	1	0	0	0
Mesembrina sp.	0	1	1	0	1	0	0	1	1	0	0	0	0
Moth sp.1	0	0	1	0	0	0	0	0	0	0	0	0	0
Moth sp.2	0	0	0	0	0	0	1	0	0	0	0	0	0
Moth sp.3	0	0	0	0	0	0	0	0	1	0	0	0	0
Moth sp.4	0	0	0	0	0	0	0	0	1	0	0	0	0
Muscoidea sp.1	0	0	0	0	0	0	0	0	0	1	0	0	0
Muscoidea sp.2	0	0	1	0	0	0	1	1	1	0	1	0	0
Muscoidea sp.3	0	1	1	0	0	1	0	0	0	0	0	0	1
Nomada sp.1	0	1	0	0	0	0	0	1	1	0	0	0	0
Nomada sp.2	0	0	0	0	0	0	1	0	0	0	0	0	0
Phyciodes sp.	0	0	1	0	0	0	0	0	0	0	1	0	0
Platycheirus sp.	0	0	1	0	1	1	1	0	0	1	0	0	0
Pyrausta orphisalis	0	0	1	0	0	0	0	0	0	0	0	0	0
Sciomyzidae	0	0	0	0	0	0	0	0	0	0	1	0	0
Sericomyia sp.	0	0	0	0	0	0	1	0	0	1	0	0	0
Sphaerophoria sp.	0	1	0	0	0	0	0	1	0	0	0	0	1
Syrphidae sp.1	0	0	1	0	0	0	0	0	0	0	0	0	1
Syrphidae sp.2	0	0	0	0	0	0	0	0	1	0	0	0	0
Syrphidae sp.3	0	0	0	0	0	0	0	0	0	0	0	0	1
Syrphidae sp.4	0	0	0	0	0	0	0	1	0	0	0	0	0
Syrphus sp.	0	0	1	0	0	0	0	0	0	0	0	0	0
Thyris sepulchralis	0	0	0	0	0	0	0	0	0	0	1	0	0
Toxomerus germinatus	0	0	0	0	0	0	1	0	0	0	0	0	0
Toxomerus marginatus	1	1	1	1	1	1	1	1	1	1	1	1	1
Villa fumicosta	0	0	0	0	0	0	0	0	0	1	0	0	0

Figure S1. Images of some of the visitors observed to pollinate *Arabidopsis lyrata*. The first row of images corresponds to members of the Hymenoptera group; the second row to the Diptera order (from left to right: *Toxomerus sp.*, *Bombylius pulchellus*, *Empis sp.*); and third row to the Lepidoptera order (from left to right: *Callophrys grynaeus, Anthocharis midea, Cupido comyntas).*

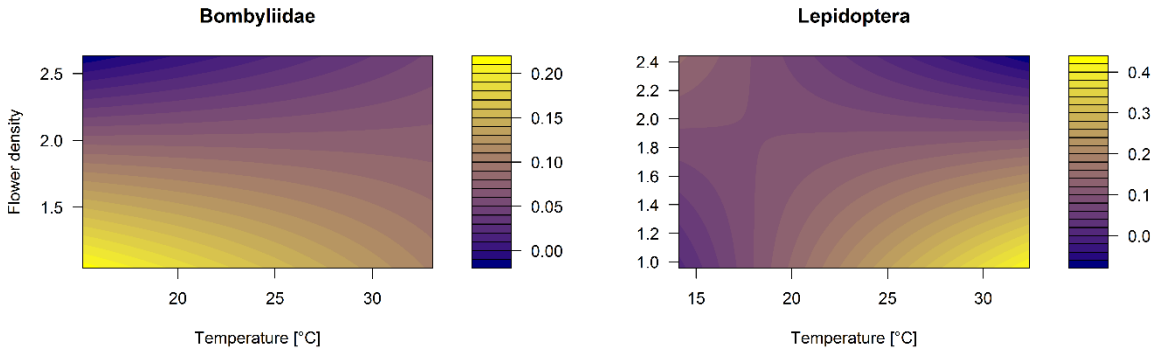

Figure S2. Relationship between temperature and flower density for Bombyliidae and Lepidoptera visitation rate for *Arabidopsis lyrata*. Figures represent a contour plot of the predicted relationship between hourly visitation rate and both, the temperature in °C, and density of flowers, n° flowers in one m^2 log_{10} transformed.

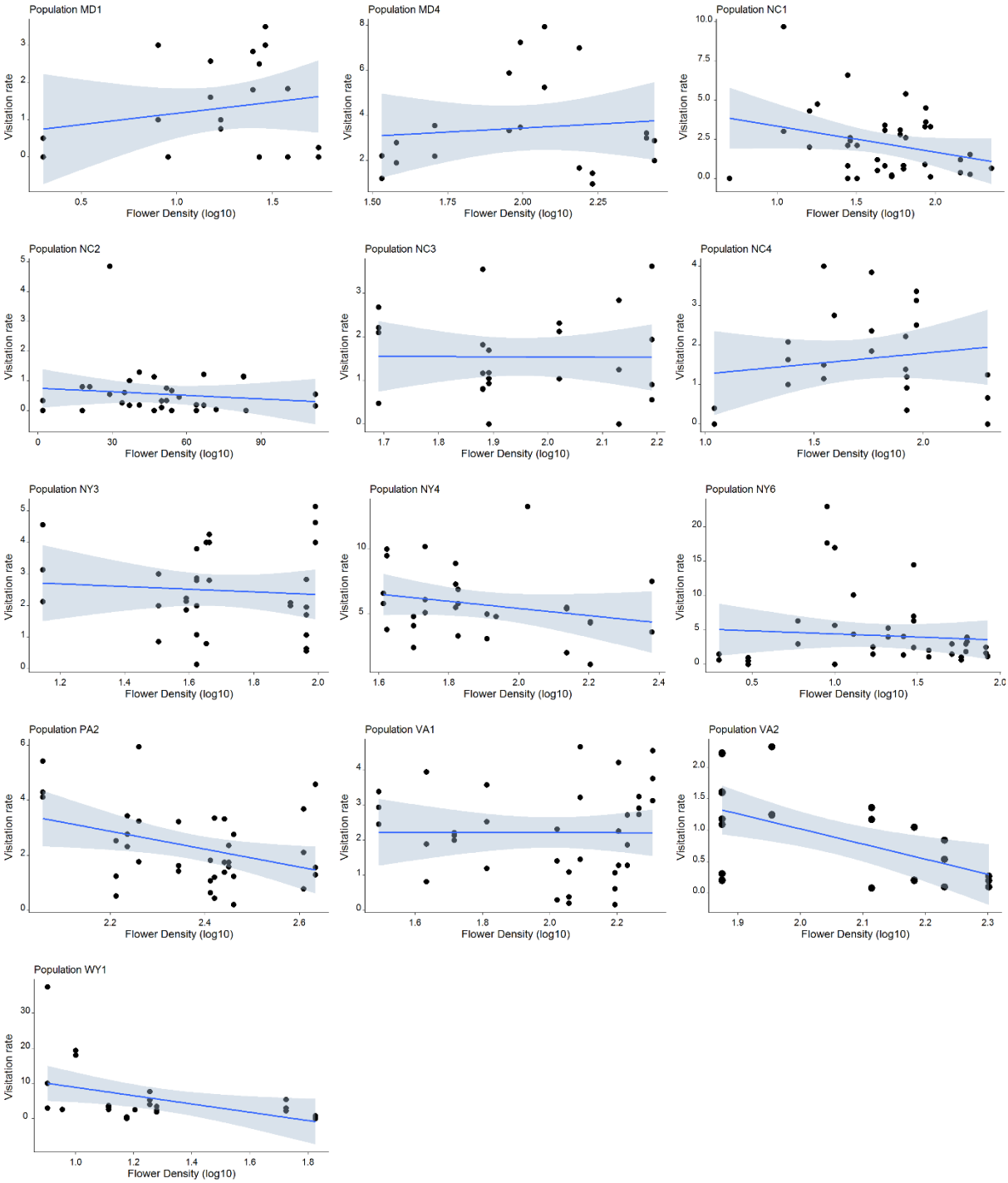

Figure S3. Visitation rate of pollinators in *Arabidopsis lyrata* depending on flower density for each population recorded. Visitation rate based on daily replicates means per population and the density of flowers log-10 transformed. The model-predicted regression line is shown in blue, with the lower and upper 95% confidence interval

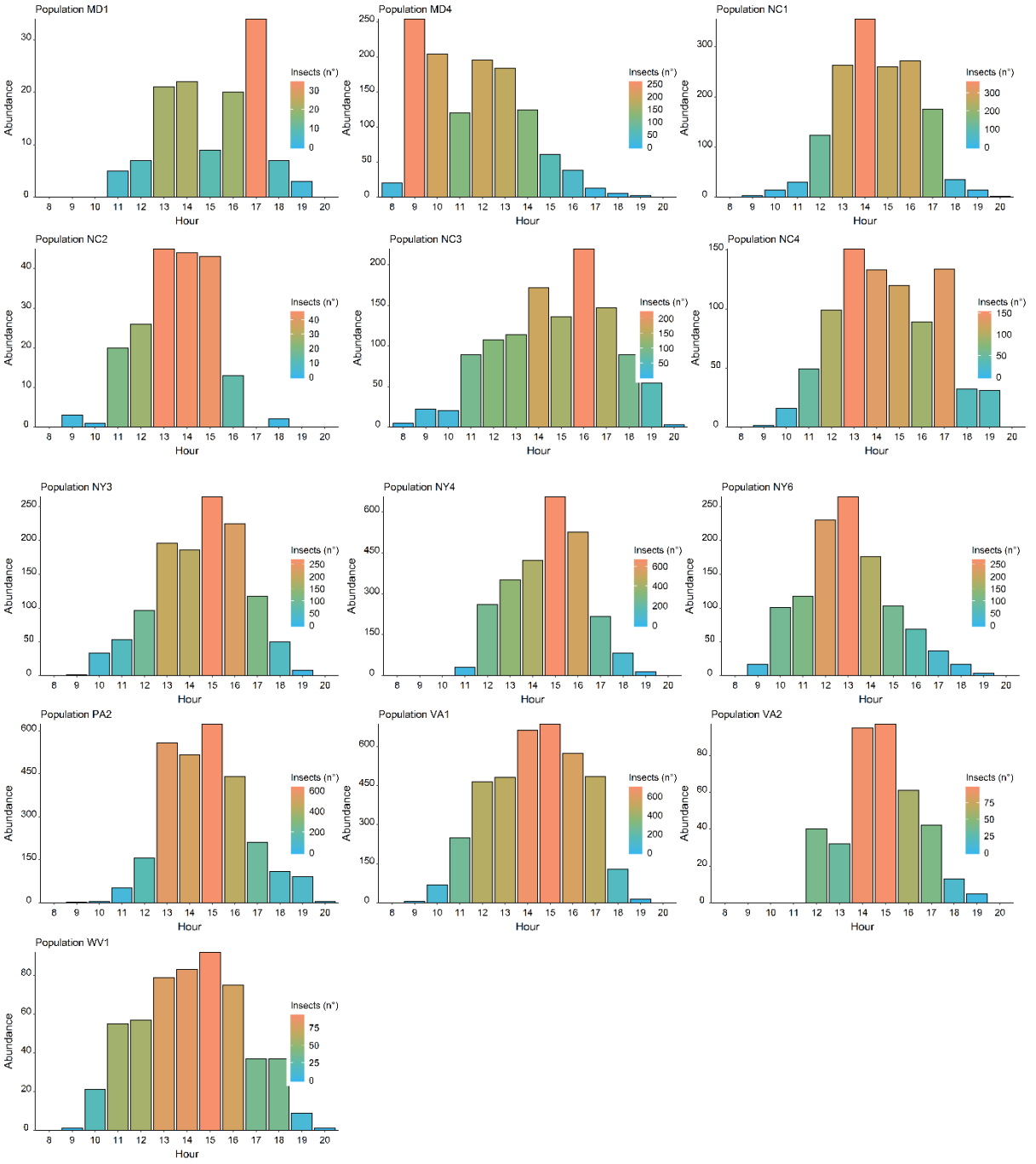

Figure S4. Relationship between insect abundance and hour of the day for each population monitored of *Arabidopsis lyrata*. The abundance of pollinators is plotted on the hour of the day at each population. The legend indicates the abundance of insects from blue to orange. A similar distribution is observed in many of the populations, with a peak of abundance falling in the centre hours of the day.

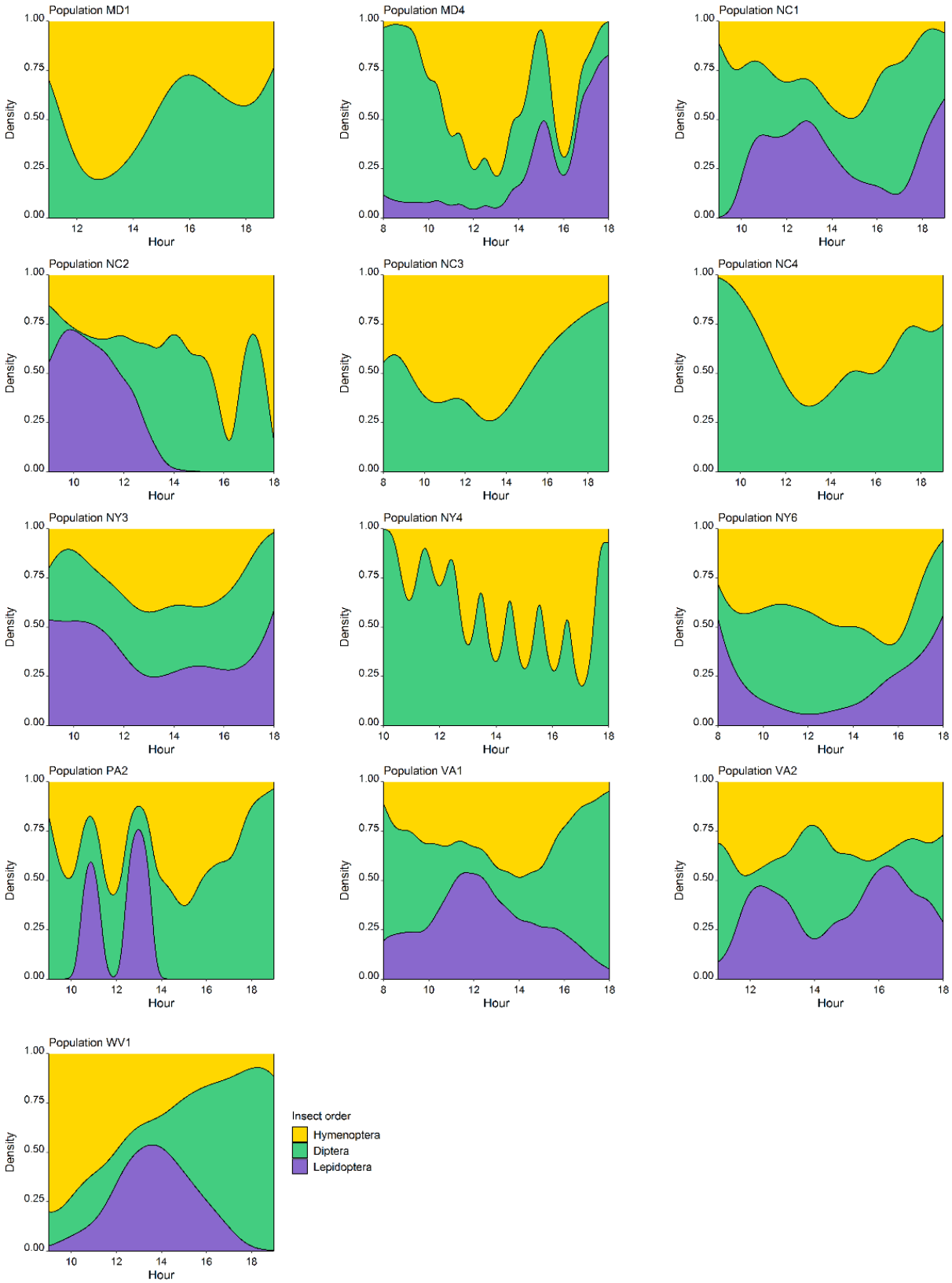

Figure S5. Density plot of insect orders across the time of day for each population of *Arabidopsis lyrata* monitored. The plots show the density of the main insect orders (Hymenoptera, Diptera, and Lepidoptera) observed across time from 8:00 to 19:59.

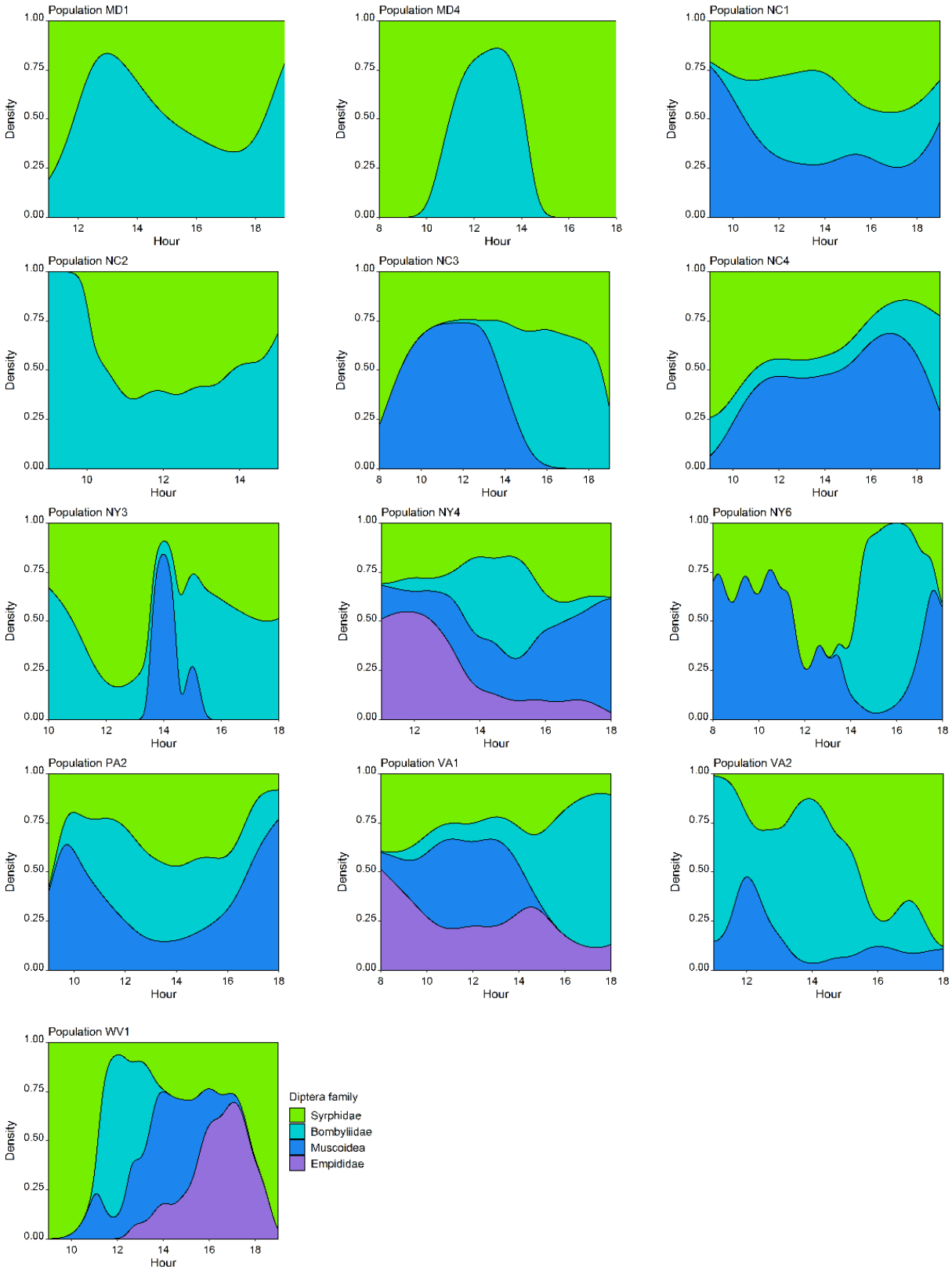

Figure S6. Density plot of Diptera taxa visiting across the time of day for each *Arabidopsis lyrata* population monitored. The plots show the density of the Diptera taxa observed across time from 8:00 to 19:59.

5. Synthesis and conclusions

5.1 Summary of findings

The main motivation for this thesis was to disentangle several factors that could potentially explain species distribution and range limits, focusing on adaptation, genetic drift, mutational load, and pollination services that influence range edges and impede the further expansion of the North American *Arabidopsis lyrata*.

In Chapter 1, I tested the role of genetic drift in adaptation across the species distribution. I performed a large-scale transplant experiment with sites within and beyond the range, and using populations covering the entire range, i.e. from the core to the edge of the distribution, the later with a history of genetic drift. Results revealed that range limits reflected niche limits of the species in the south, as population performance beyond the southern range limit was non-persistent. However, northern range limits did not reflect niche limits, which may be a result of recent global warming. Furthermore, a signature of climate adaptation was revealed across populations, but, the magnitude of adaptation was lower in populations with a history of genetic drift typical for range edge populations. These results support that adaptation at geographic range edges is constrained due to genetic drift, which might explain why the further expansion of the species is not possible.

In Chapter 2, I showed experimentally that both leading and rear edge populations additionally suffered from the increased expression of mutational load. Expressed mutational load was estimated by the heterosis effect of between-population crossing. Heterosis increased in those populations with greater genomic estimates of mutational load, with longer expansion distance from the core, and in those with a selfing mating system (predominantly located at the distribution edge), aggravating the negative effect of load at range edges. Interestingly, the magnitude of load expression increased over the lifetime of the organism. This thesis chapter unequivocally showed that the accumulation of mutational load is a second

genetic problem marginal populations have and that impedes further expansion.

In Chapter 3, I focused on the potential role of pollinators at the range edges and the mechanistic processes at play. I monitored 13 natural populations of *A. lyrata* across a latitudinal gradient from northern to southern range limits. Results included that pollination services declined from the core to the southern range edge but not to the northern range. Plant populations with greater census size had also greater rates of flowers to be visited. However, visitation rate declined when flower density was high. This could suggest that those populations with low pollination services might tend to produce a greater amount of flowers to increase the attractiveness to pollinators, while big populations might not necessarily need to increase attractiveness as visitation rate is already high. Additionally, the diverse number of insect taxa observed supports the idea that the *A. lyrata*-pollination network is a generalist system that brings ecological flexibility of resources for the organisms involved and which guarantees survival in case one of the species involved goes extinct.

In Chapter 4, I focused on the variation of pollination services across time and space on various scales. Pollination services are far from constant across time and space, which has been widely observed in previous research. My results supported the general idea that pollinator activity is greater during the central hours of the day, coinciding with the warmer hours. A pattern of temporal niche partitioning between the different insect groups was not revealed. However, some pollinators were more tolerant under certain temperature regimes. While bees preferred the central hours of the day, syrphids were more tolerant to visit during the morning hours; and Muscoidea to the warmest temperatures of the day. The difference in visitation rate could not be explained by the density of flowers, however, the different insect groups exhibited distinctive preferences. Bees and butterflies were attracted toward high-density flower patches, while other groups did not show such a pattern, or even a negative correlation, as was found for Bombyliids. The temporal and spatial variation may be

beneficial as it provides resource flexibility for the plants and pollinators and which is likely to ensure their persistence.

5.2. Ecological and evolutionary implications

Chapter 1 reported on the reduced signature of climate adaptation in populations with a history of small population size. This insight has important implications for our understanding of current species distribution and conservation. The magnitude of climate adaptation seems to be driven by a combination of local ecological conditions imposing selection and a history of genetic drift, which at the end will decide the fate of the species under current and future conditions. In our study, plant life-time fitness at and beyond the southern range edge was low, and population growth not high enough for long-term persistence. Furthermore, climate adaptation was constrained due to the low population size of range edge populations. The two insights suggest that southern range edges in the species may remain stable under no global warming. But under global warming, the species range is likely to lose terrain in the south, as niche limits will be met further north, and because there is little scope for adaptation to a warmer climate due to small population size. In contrast, many northern range edges may not be niche-limited anymore, and therefore it could be that northern ranges may expand beyond the current range under global warming if general habitat is available.

Chapter 2 illustrated that a history of small population size can constrain range expansion also via mutation accumulation. This load was found to be larger in populations of range edges compared to core populations. Under climate warming, high mutational load in marginal populations could impede the expansion of the organisms into newly available habitats. Therefore, range expansion by northern-edge populations may not happen or be slow despite the availability of generally suitable habitat toward more northern areas. The detection

that population history impacts population persistence at range edges implies the need for the integration of evolutionary history into biodiversity conservation management. In the same study, between-population crossing was however found to help overcome mutational load and increase life-time fitness. This result suggests that gene flow, either natural or assisted by humans, could alleviate the situation for range edge population, particularly in the north where conditions may improve for the species.

In Chapter 3, the differences in pollination services across the latitudinal gradient of *A. lyrata* distribution showed parallel results to those found in Chapter 1. Southern range limits are not only niche limits because of a lack of suitable climate, but southern conditions are also not great in providing pollination services. If the pollination-service persists, this would have important consequences for the maintenance of the population size and even lead to an Allee effect in the area of the current edge. The positive relationship between population census size and pollination rate suggested that large populations have a greater number of visits. My results also highlight the importance of diversity in flower resources in the ecosystem to attract a greater diversity of pollinators. Additionally, the study supports the idea that many plant-insect interactions are generalist systems, which has important ecological consequences as it brings greater flexibility in terms of resources and pollen transfer for the organisms involved. Plant diversity seems to maintain pollinator diversity, and the relationship is likely to be reciprocal.

Spatial and temporal variation in pollination services is an important feature of the plant-pollination interactions that bring flexibility to the network. In Chapter 4, I found that there is no reason to think that all insect pollinators should have similar temperature optima and similar preferences toward larger flower display. It seems clear that the temporal variation on a small scale, on an hourly basis, is mostly explained by temperature regimes. However, tolerance and activity of pollinator guilds vary between the different groups. This implies that

under a scenario of climate warming, some insect groups might be more vulnerable than others and their contribution as pollinators will change in the near future. I found that some pollinators showed positive correlations toward high-density patches of flowers, while others did significantly preferred patches with few flowers. This highlights the importance of some insect groups for pollen transfer and the expansion at the range. For example, Bombyllids might be especially important under fragmented habitats or at the leading edge of the population, where the density of flowers is low and not many pollinators feel attracted.

5.3. Future considerations

This research highlighted some important considerations for the study of species range limits in general. The use of a two-year common garden experiment monitoring plant performance of 20 populations across and beyond the species distribution is the key to fully understand range limits and climate adaptation patterns. Additionally, the identification and quantification of pollinators using time-lapse cameras were extremely valuable to understand the variation in pollination services across the latitudinal gradient. It allowed us to have complete samples over different temporal scales (along the day, among days, and years) and across space to fully cover the spectrum of pollinators' niches. This research has expanded our understanding of how species distribution limits establish and what factors drive range limits. However, like all research, new questions have risen and novel gaps of knowledge need to be filled.

Firstly, more research is needed at the northern range of *A. lyrata* to comprehend why the species is not niche-limited, and whether the current climate warming is shifting the habitat suitability at the northern range edge. Furthermore, an additional transplant experiment might be desired at the western cluster of *A. lyrata* to deeply explore what causes the range limits in in Ontario and Missouri. It might be that what is limiting at the eastern side of the

range does not limit at the western side as different ecological factors might set the western range limits. Furthermore, as the plant is a generalist in terms of pollinators, transplant experiments beyond the current range assessing pollinator services would help to understand whether populations would have potential pollinators under an expansion scenario.

For the study of pollination biology, time lapse-cameras are a particularly powerful tool to obtain complete records of the pollinator network across different time and space scales, however, they imply a lot of effort in monitoring and spotting pollinators in the video frame. A high-resolution software would be highly valued to identify and quantify pollinators to avoid manual observations. Additionally, image quality of the time-lapse cameras did not allow always to identify low taxonomic groups. Identifying pollinators to the lowest unit possible, varied depending on the insect order. For Lepidoptera identification to the genus or species level was possible; for the Diptera groups, I identify some genera but mostly family groups, while for Hymenoptera only morphotypes were possible. Deeper taxonomic identifications might reveal a clearer picture of interspecific niche partitioning, on specific temperature tolerances and/or display preferences.

All visitors were quantified equally to their effectiveness as pollinators, however, not all visitors have the same contribution to the reproduction of the plant. Some pollinators landed gently on the flower and might not influence actively the reproduction and pollen transfer (this is the case of syrphids), while others actively collected and rubbed over the reproductive organs (for example bees). Information on specific pollinator species and their effectiveness as pollinators might be needed to quantify their relative importance in the interaction network. Even though the number of populations and record hours for Chapters 3 and 4, is one of the most extended studies of pollination records, the great variation among populations suggests that pollination services might be very different in populations at the

western cluster. Exploring the pollination network in western populations would contribute to having complete comprehension of the entire pollination network.

Particular interesting would be also to assess the differences in flower attractiveness between populations across the distribution range. It is known that *Arabidopsis lyrata* produces nectar and volatile compounds to attract pollinators, however, there is no research assessing the differences in attractiveness compounds across the range of the distribution, as far as I know. It might be that range edge populations produce less volatile compounds due to the lack of resources; or on the contrary, they might invest more energy in nectar and volatile compounds production due to the lack of pollinators and then guarantee the population growth.

5.4. Concluding remarks

Evolutionary theory on species distribution limits along environmental gradients advocates a strong effect of the steepness of gradients to range limits. The main motivation for this thesis was to disentangle all the factors that could potentially explain species distribution limits (Fig. 1). I considered several factors such as adaptation, a history of genetic drift, mutational load, and pollination services that could potentially influence species range edges and impede further expansion. The research provides new insights into the effects of a history of genetic drift, of reduced adaptation and mutation accumulation to range limits. Results corroborate that southern range limits coincide with niche limits, but that this is not the case for northern range limits – anymore. I found support for the prediction that populations are climatically adapted to their local habitats, however, populations at the range edge with a history of genetic drift seem to be less well adapted (Fig. 2). This lack of genetic adaptation and the accumulation of deleterious mutations due to the long term small population size seems to shape the edges of species ranges. Furthermore, pollinator services were lower at southern

range edges but not in the north (Fig. 2). The pollination network was found to be a generalist system, which provides wider ecological flexibility for all organisms involved. The fine-scale variation in pollinator activity is well explained by temperature regimes, however, the spatial variation might be related to preferences in local flower density which is taxa specific.

This research contributes to a better understanding of the species range limits and the interplay of diverse aspects involving past range expansion, genetic consequences of small population sizes, genetic load, the ability to adapt, and the interaction network with pollinators. This implies that species distribution models along environmental gradients should incorporate other factors rather than only abiotic gradients, such as population size and density, genetic drift and its consequences, and their complex interactions. This would increase our understanding of current species ranges limits and how species will respond under current global warming.

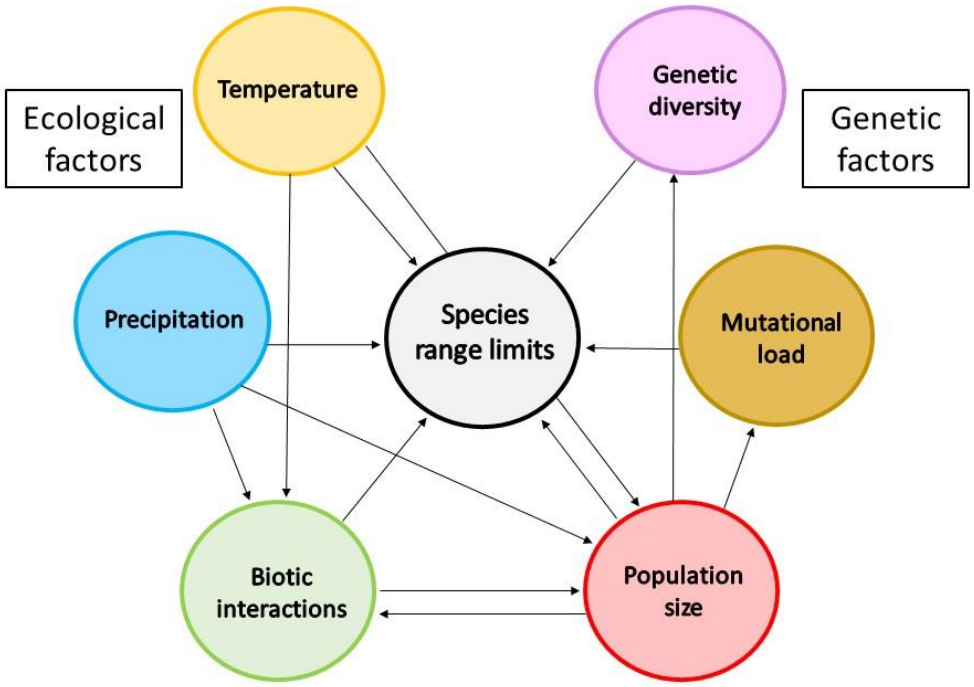

Figure 1. Representation of the ecological and evolutionary factors that could explain the species distribution limits in general.

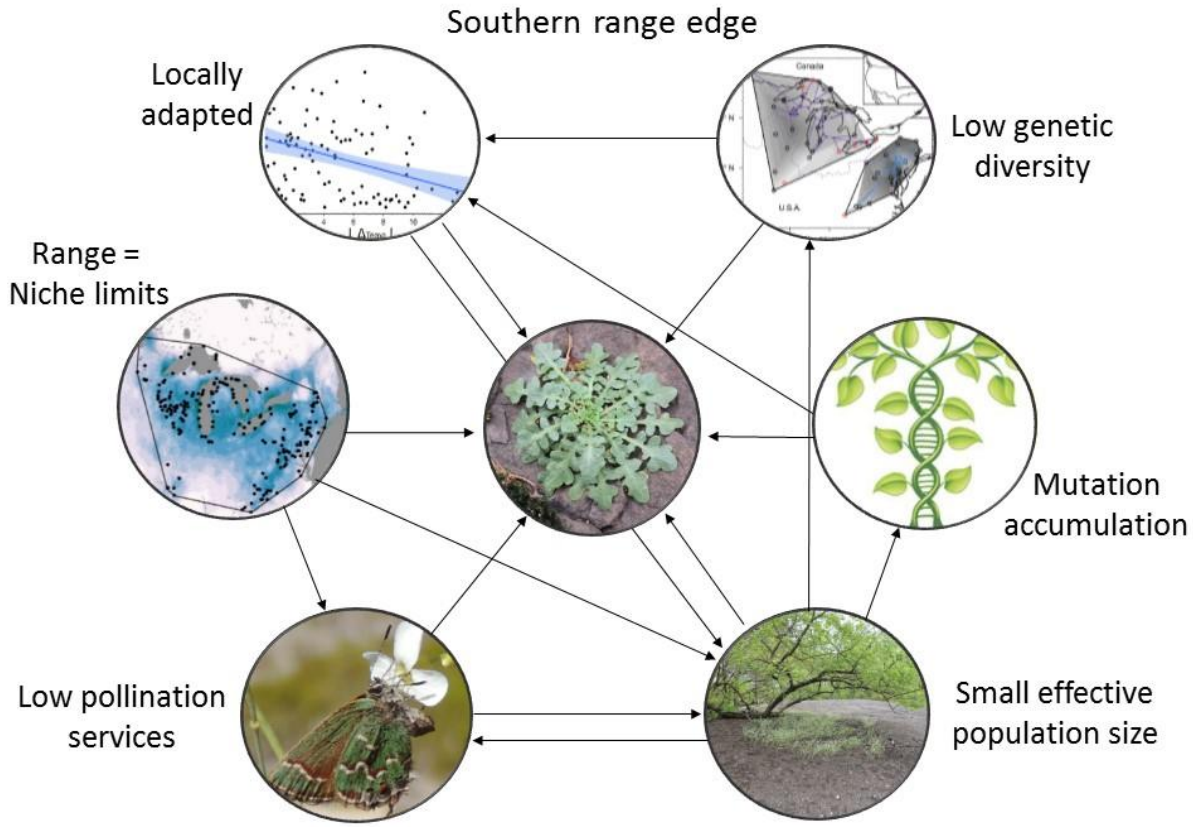

Figure 2. Representation of the ecological and evolutionary aspects found to be important for the range limits of the North American *Arabidopsis lyrata*.