Title:

The spatial scale of adaptation in a native annual plant and its implications for responses to climate change

Running title: Spatial scale of adaptation in common ragweed

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Author contributions
AJG, DAM and PLT conceived the experimental design and questions. AJG conducted seed and data collection, and contributed to data analysis and interpretation. JWB conducted the data analysis and interpretation. AJG and JWB drafted the initial version of the manuscript, and DAM and PLT contributed to later versions of the manuscript.

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Data archiving: Data will be archived on FigShare, and all data and code has been uploaded as supplemental files for review.

Abstract

Spatial patterns of adaptation provide important insights into agents of selection and expected responses of populations to climate change. Robust inference into the geographic scale of adaptation can be gained through reciprocal transplant experiments that combine multiple source populations and common gardens. To quantify the scale of local adaptation and examine the consequences of a warming climate on the species' range, we performed reciprocal transplant experiments at four common gardens with 22 populations sampled across a ~1200 km latitudinal gradient within the native range of common ragweed, *Ambrosia artemisiifolia.* We found evidence of local adaptation at the northernmost common garden, but maladaptation at the two southern gardens, where more southern populations outperformed local populations. Overall, the spatial scale of climate adaptation was large — at the three gardens where distance between source populations and gardens explained variation in fitness, it took an average of 820 km for fitness to decline to 50% of its predicted maximum. However, fitness declined more sharply with distance in southern transplant sites. Taken together, these results suggest that climate change has already caused maladaptation, especially across the southern portion of the range, and may result in northward range contraction over time.

Key words: local adaptation, range shifts, reciprocal transplant, maladaptation

Introduction

The extent to which populations are locally adapted depends on a balance between environmentally-dependent selection and gene flow (Linhart and Grant 1996). Populations separated by short geographic distances are likely to experience high gene flow, which is expected to constrain local adaptation (Slatkin 1987; Lenormand 2002). However, nearby populations are also likely to share similar environments and experience more similar selection. Thus, nearby populations may not be expected to exhibit adaptive differences even in the absence of gene flow. As the distance between populations increases, gene flow is likely to decrease and environmental differences are expected to increase. Accordingly, the extent of adaptive differentiation may be expected to increase with geographical distance among populations (Montalvo and Ellstrand 2000; Joshi et al. 2001). However, this expectation assumes that environmental differences and geographic distances are strongly correlated, which may not often be true. For example, selection can vary dramatically over only a few meters (e.g. serpentine soils, heavy metal contamination), and can result in fine-scale adaptive differences (Antonovics and Bradshaw 1970; Janson 1983; Sambatti and Rice 2006).

The most powerful method to identify patterns of local adaptation is the common garden transplant approach, where multiple source populations are planted into one site and performance is compared among populations. Although many transplant experiments have been conducted (e.g., Antonovics and Bradshaw 1970; Galloway and Fenster 2000; Geber and Eckhart 2005; Becker et al. 2006; Brachi et al. 2013; Wilczek et al. 2014; Anderson et al. 2015; O'Brien et al. 2017; Wright et al. 2018; Bachmann and Van Buskirk 2021), few assess lifetime fitness along a broad environmental gradient among populations sampled from a broad geographic area (but see Colautti and Barrett 2013; Wilczek et al. 2014). The use of multiple common gardens across the

range of a species combined with extensive population sampling is required to develop quantitative, as opposed to qualitative, estimates of the spatial scale of adaptation. Furthermore, examining organismal lifetime fitness provides a more direct measure of adaptation than components of fitness such as survival, or proxies of fitness such as growth. With a multi-garden transplant experiment recording lifetime fitness in many source populations, we can begin to evaluate the geographic scale of local adaptation, the extent to which geographic and environmental distance explain fitness variation, and the extent to which inferences of local adaptation depend on the environment in which experiments were conducted.

Characterizing the spatial scale of adaptation and the relationship between fitness and distance (between a source population and common garden) for populations sampled across a climate gradient can provide insight into factors that may facilitate or constrain range shifts in response to climate change. Evidence is accumulating that climate change is disrupting patterns of local adaptation (Anderson and Wadgymar 2020; Bontrager et al. 2020) and has already resulted in range shifts for some species (Chen et al. 2011; Lenoir et al. 2020). Experiments that assess fitness of populations sampled from across a climate gradient (e.g., latitude) can provide insights into the scale of local adaptation, scale that has direct implications for response of populations to climate change. A shallow relationship between fitness and geographic distance would suggest that species will be relatively unaffected by climate change, whereas a steep relationship between fitness and geographic distance would suggest that climate is an important driver of adaptation and populations are thus likely to be strongly affected (Fig. 1a). Such experiments can also uncover altered patterns of local adaptation due to climate warming that has already been realized, for instance if populations from warmer regions have higher fitness than local populations (Gorton et al. 2019; Bontrager et al. 2020; Fig. 1b). Furthermore, if populations

are sourced from regions colder than the common garden, the transplant experiment can be thought of as a space-for-time substitution (e.g., Etterson and Shaw 2001). By evaluating the performance of "cold" populations in a warmer locale, one can predict fitness of these populations in their home sites with future warming. Alternatively, if populations are sourced from regions warmer than the common garden, transplanting into a more northern or cooler garden can mimic potential future migration as populations track shifting climatic isotherms. In this case, fitness in the common garden would reflect adaptation to potentially important non-climatic aspects of a future environment that will likely not shift with climate change, such as day length or soil type.

In an effort to identify drivers of local adaptation, differences between source populations and common gardens is often characterized using geographic distance (e.g., Becker et al. 2006), temperature difference (e.g., Bontrager and Angert 2019), or multivariate environmental distance (e.g., Wright et al. 2018). Geographic distance is easy to measure and interpret, but is not a factor that drives local adaptation. Instead, it effectively integrates multiple (known and unknown) environmental variables that do drive adaptation, given that environmental variables are often spatially autocorrelated. For instance, in the case of populations distributed across large geographic scales, geographic distance might capture variation in climate and photoperiod, both strong determinants of fitness in at least some plant species (e.g. Lacey 1988; Li et al. 2003; Fournier-Level et al. 2011; Keller et al. 2011). However, geographic distance is usually calculated as the absolute, Euclidean distance between locations, and therefore does not differentiate between directional differences that may be important. For instance, transplanting a population 200 km north will likely have different effects on fitness than transplanting that same population 200 km south. Incorporating directional geographic distance (e.g., latitude / longitude

of source population - latitude / longitude of common garden) will likely be especially important for investigating patterns of local adaptation in the context of climate change.

We used a reciprocal transplant experiment of a North American annual plant, *Ambrosia artemisiifolia* (common ragweed), to quantify the strength and scale of local adaptation and explore the consequences of a warming climate across the species' geographic range. The experiment included four common gardens, in which we planted individuals from 22 populations collected across a ~1200 km latitudinal gradient. Our main questions were:

1) What is the scale of local adaptation to climate in ragweed?
2) Do patterns of local adaptation differ across the species' range?
3) Which life history stages underlie local adaptation and how do these vary across latitude?
4) How will populations from different regions respond to conditions that simulate future climate change?

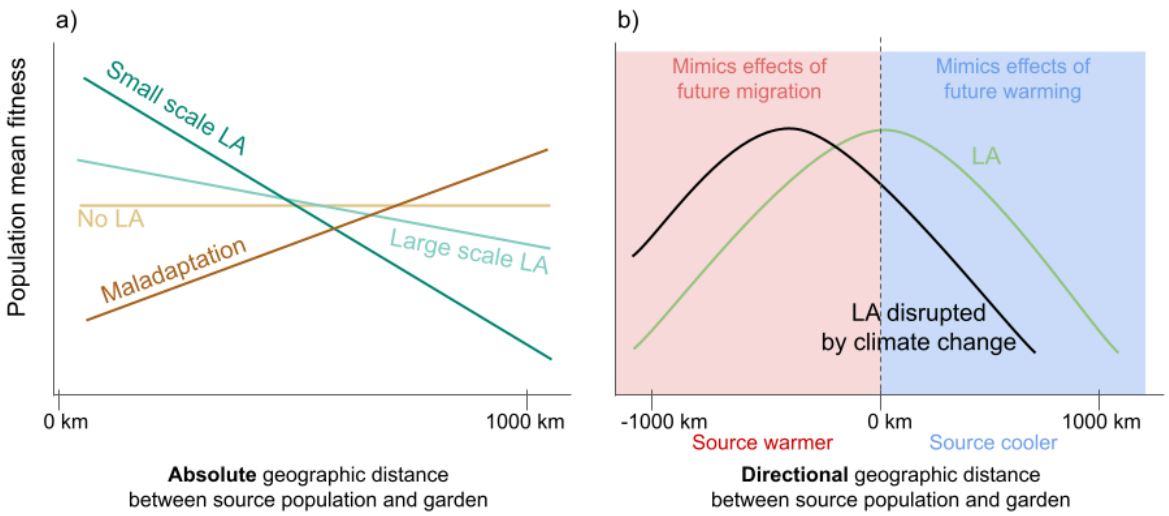
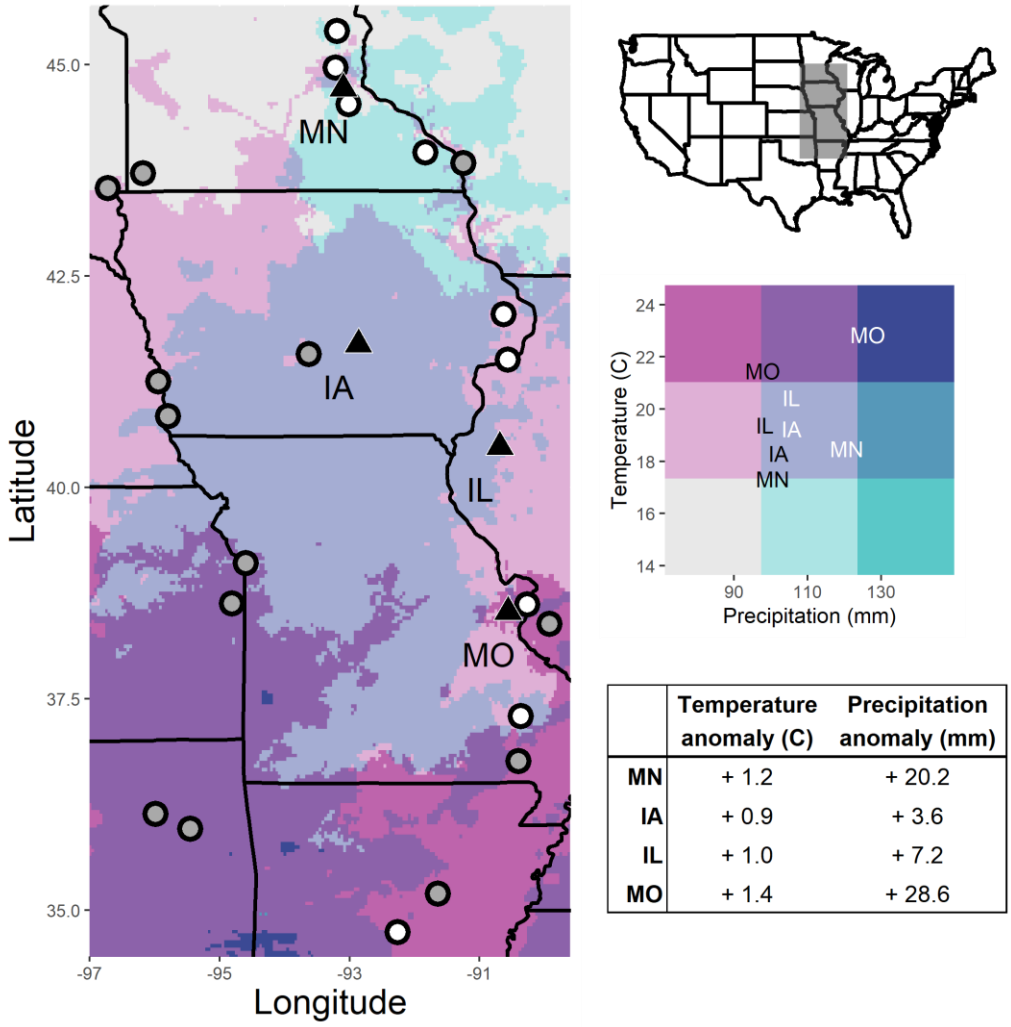


Figure 1. The geographic scale of adaptation can be estimated using regression analyses. **a)** a steep slope is indicative of a small spatial scale of adaptation, whereas a shallow slope is indicative of a large scale of adaptation. Such analyses could also show no relationship between fitness and distance (and thus no evidence for local adaptation), or a positive relationship, which would indicate local maladaptation. **b)** shows how regressing fitness on a directional geographic distance metric (such as latitudinal distance, calculated as *source population latitude - common garden latitude*) can provide insight into whether local adaptation has been disrupted by contemporary warming, and offers predictions for population performance with future warming and migration. Transplanting populations into gardens north of their location tests adaptation to sites potentially occupied after future migration, while transplanting populations into gardens south of their location tests adaptation to warmer temperatures expected to reach those populations in the future.



	Temperature anomaly (C)	Precipitation anomaly (mm)
MN	+ 1.2	+ 20.2
IA	+ 0.9	+ 3.6
IL	+ 1.0	+ 7.2
MO	+ 1.4	+ 28.6

Figure 2. Map of locations of the 22 source populations (circles) and four common gardens (triangles). White circles show the nine focal populations for which full lifetime fitness data was collected; grey circles show the remaining 13 populations. Bivariate climatic regions are shown based on growing season (May - October) weather, averaged over years 1986-2016. Garden names are plotted on the color legend according to their historical climate averages (1986-2015, black text) and experiment year weather (2016, white text). Table shows how weather during the experiment year (2016) differed from the long-term average (1986-2015).

Methods

Study system

Common ragweed is a self-incompatible (Friedman and Barrett 2008), monoecious, wind-pollinated annual plant (Jones 1936; Essl et al. 2015) widely distributed across North America and invasive on several continents (Genton et al. 2005; Chauvel et al. 2006; Xu et al. 2006). It is a ruderal plant that is often abundant in open, disturbed habitats such as river banks, roadsides, edges of agricultural fields, and urban areas.

A. artemisiifolia is a summer annual that typically germinates in late spring (May – June) and flowers in late summer or early fall (August – Sept). The onset of frosts in the fall terminates the growing season (Essl et al. 2015). In North America, the species' abundance increased dramatically as agriculture spread across the continent (Grimm 2001; Martin et al. 2014). Today, common ragweed is most abundant in the eastern and midwestern United States and Canada, but is distributed more broadly across both countries (Kartesz 2013).

The transition to reproduction is cued by shortening day lengths after the summer solstice. Staminate capitula (*i.e.* male flowering heads) are found in spike-like racemes, which produce pollen that is one of the primary causes of summer and fall allergic rhinitis (Lewis et al. 1983; Frenz 2001). Pistillate capitula (*i.e.* female flowering heads) are found in axillary clusters below the male flowers; each individual flower develops into an achene (a small, single seeded fruit) that readily falls off the plant once ripe. Hereafter we refer to these groups of achenes as 'fruits'.

Latitudinal clines in flowering time are present in both the native and introduced ranges, with plants from more northern latitudes flowering earlier (Hodgins and Rieseberg 2011; Leiblein-Wild and Tackenberg 2014; Gorton et al. 2019). These results suggest that there may be

local adaptation to climate and photoperiod in *A. artemisiifolia* at geographic scales of hundreds of kilometers. Population genetic data also revealed weak population structure and evidence of loci contributing to local adaptation to climate among North American populations of ragweed (Hämälä et al. 2020). By contrast, phenotypic differentiation among plants sampled from 1 – 3 km distances (Kostanecki et al. 2021), suggests that adaptive divergence may occur at fine spatial scales.

Seed collections

During October 2015 – January 2016, we collected seeds from 22 populations of *A. artemisiifolia* across a region spanning 11° latitude (~1200 km) and 7° longitude (~550 km) (Table S1, Figure 2). Sampling locations were an average of 290 km apart along two latitudinal transects. At most latitudes, we sampled two populations separated by 50 – 60 km. The populations were collected from a range of habitats, including abandoned lots in urban areas, along roadsides, river edges and parks. At each sampling location we collected seeds from 16-25 maternal plants, each separated by at least 3 m.

Reciprocal transplant experiment

In Spring 2016, we planted four common gardens (hereafter 'gardens') that were separated by an average of 260 km (range: 218 – 338 km) and differ in annual temperature, precipitation, and photoperiod (Figure 1). The gardens were in Minnesota (MN), Iowa (IA), Illinois (IL), and Missouri (MO) (Table S2 for exact locations). The soil in all gardens is a silt loam mixture.

Between May 15 and May 30, 2016, we planted the four gardens, starting at the southernmost site (MO) and ending in the northernmost site (MN). The timing of planting approximately matched the germination timing of natural populations at each latitude (Gorton, personal observations). Prior to planting, each garden was sprayed with glyphosate and tilled, creating an environment similar to the disturbed, low-competition habitats where *A. artemisiifolia* is commonly found. The seeds were planted in a randomized complete block design, so that each block contained seeds from each population (22 populations × 25 plants per population = 550 plants/ garden). For each source population, we pooled an equal number of seeds from 12 – 25 maternal plants (Table S1).

A. artemisiifolia has the potential for long-term dormancy, with seeds in the seed bank remaining viable for many years (Toole and Brown 1946). To minimize confusion between experimental plants and those that emerged from the seed bank, we used a ProPlugger (ProPlugger, NC) to remove a soil plug (5 cm x 5.5 cm) at each planting spot. The holes were then filled with Berger germination mix (Berger, Quebec). Prior to planting, all seeds were stratified in moist silica sand in the dark at 4°C for 10 weeks to break dormancy (Willemsen 1975). At each planting spot, we planted 2 – 4 stratified seeds directly into the ground and watered the seeds immediately after planting. Each garden had 55 rows and 10 columns of plants, planting spots were spaced 15 cm apart along each row, and the spacing between columns alternated between 30 and 80 cm. Four weeks after planting, plants were thinned to a single seedling per planting spot. Throughout the growing season, all gardens were regularly weeded to reduce competition from non-experimental plants.

Fitness components

We visited each garden 4, 8, 12, and 16 weeks after planting, and at the end of the growing season. During each week, we documented survivorship and scored each plant for overall reproductive stage, male flowering stage and female flowering stage (scoring categories presented in Table S3). We estimated female fitness at each garden by counting the number of mature fruits on 12-15 randomly selected plants from each of 9 of the 22 populations (108-135 plants per garden). These nine populations span the full latitudinal range of the source populations (Table S1). Due to the distance between gardens, the very high fecundity of individuals, and the narrow time window in which data needed to be collected, it was not possible to quantify female fitness for every plant. For those plants for which we estimated female fitness, we counted the number of fruits on every fourth branch, starting with the lowest branch, and moving up towards the apical meristem. We subsampled branches (ca. 20 – 50 / plant) in this way to account for potential variation in allocation to female reproduction across plant development. We multiplied this number by four to get a whole-plant estimate of female fitness. Fruits were considered mature if at least one achene in the axillary cluster was brown. Plants with only immature green fruit were given a score of zero. We collected data on female fitness (number of mature fruits produced) at both 16 weeks after planting and at the end of the growing season to capture the range in life span of the different populations and the range in length of the growing season across gardens. We counted fruits on the same individuals at both visits, and where the fruit counts differed across visits, we kept the higher value. One person (AJG) counted all fruits.

Statistical analyses

We used linear regression models to estimate how the relationship between mean population fitness (measured as mean individual fruit count) and distance (absolute geographic, directional geographic, and climatic) varied among common gardens and to identify specific life history stages that showed the strongest signal of divergence among populations. Analyses were conducted in R and all data and code to replicate the analyses are available at FigShare (will be posted; for review, data and code are provided as supplemental files).

Environmental distances

We contrasted two metrics representing the geographic distance between each source population and common garden: absolute geographic distance and directional geographic distance. Absolute geographic distance is most often measured as the straight-line, Euclidean distance between two points. This common measure of geographic distance, however, fails to account for directionality — e.g., the environment a fixed distance north of a garden is likely to be quite different from the environment found that same distance south of a garden. For this reason, we calculated *directional* geographic distance measures. These were simply differences in latitude and longitude between source populations and gardens, hereafter referred to as directional latitudinal and longitudinal distance. To make directional geographic distance directly comparable to absolute geographic distance, we decomposed absolute geographic distance into the absolute values of latitudinal and longitudinal distances between populations and gardens. Results from analyses using straight-line absolute geographic distance (i.e., not decomposed into absolute latitudinal and longitudinal distance) were qualitatively similar to results from the two-variable absolute distance metrics (Table S5).

Another option is to characterize the distance between source populations and common gardens in terms of explicit environmental variables, such as differences in temperature and precipitation. Thus, we also calculated climatic distance using monthly mean temperature and monthly total precipitation data from 1986-2016 extracted from PRISM (PRISM Climate Group 2021). We extracted monthly climate data for the growing months (May – Oct) for each source population from 1986 – 2015, and for each garden in 2016 (the experiment year). We then calculated mean monthly temperature and precipitation for each year as the average across the six growing months. For source populations, mean temperature and precipitation was averaged over years 1986 – 2015. Climatic distance between source populations and gardens was defined by differences in temperature and precipitation. Temperature difference was calculated as the difference between a source population location's long-term mean temperature and the experiment year (2016) temperature of the common garden. Precipitation difference was calculated in the same fashion. Climatic distance was thus directional, not absolute.

Predictors of fitness

We used the life history data (stage at 12 weeks, female reproductive stage at 12 weeks, height at 16 weeks, fruit number) collected on plants from the 9 focal populations to build predictive models of fitness based on individual phenotypic data. We then used these models to predict fitness of the individuals from the remaining 13 populations, for which we had all life history data prior to fruiting (SI: Predicting fitness). Results from regressions of *fitness ~ distance* using data from either the 9 or all 22 populations were very similar (Supplemental Information, predicting fitness; Fig. S3); we present results using the 22 populations because these capture a wider geographic sampling of populations and increased statistical power.

We used linear regression to evaluate the relationship between our environmental distances (absolute geographic, directional geographic, and climatic) and variation in fitness among populations at each garden. Specifically, we used linear regression to regress population mean fitness (mean fruit set of all individuals in a population) on distance metrics separately (e.g., *lm(fitness ~ directional latitudinal distance + directional longitudinal distance)*. We then estimated the proportion of among-population variance explained by each of the distance metrics using ANOVA with Type II SS. Because the *a priori* expectation for the relationship between fitness and our directional distance metrics (directional geographic and climatic) is quadratic (Fig. 1b), we compared linear to quadratic models. ANOVA did not support inclusion of quadratic terms at any garden (all $P > 0.08$ for F tests comparing models with only linear terms to models with both linear and quadratic terms).

Conditional fitness component analyses

The above analyses identify the relative ability of different distance metrics to explain fitness variation, but they do not identify the life history stages that underlie adaptation. To identify those stages, we analyzed fitness components (probability of survival to 4 weeks, probability of fruiting, and number of fruits) to identify which life history stages most strongly drive overall lifetime fitness. For the subset of plants where fruits were not counted in the field, we used the predicted values from phenotypic models (same as in lifetime fitness analyses, SI: Predicting fitness). For probability of mature fruits, all individuals that were scored as having at least one mature fruit were assigned a value of 1, and others were assigned a value of 0. Analyses were conditional such that individuals were only included in the analysis if they successfully reached the previous life history stage (i.e., only plants that survived to 4 weeks are included in

the analyses for probability of fruiting; number of fruits were modeled only for plants that produced fruits). We used logistic regression on population-level data (*e.g.* each data point was number of successes and failures per population) to estimate the influence of the distance metric that explained the most variation in lifetime fitness regressions on probability of early survival and probability of fruiting; *glm(cbind(successes, failures) ~ distance, family = binomial)*. We used linear regression on population means to estimate the influence of the same distance metric on the number of fruits; *lm(number of fruits ~ distance)*.

Results

Fitness across gardens

Growing season temperature and precipitation were higher than the historical average at all gardens (Fig. 2). Ragweed fitness differed starkly among gardens, with the lowest fitness (mean of 173 fruits per plant) at the northernmost garden, MN, and highest fitness (968 fruits per plant) at IL, the garden near the center of the sampled populations (Fig. 3). There was considerable among-population variation in fitness at each of the four gardens. However, we found evidence of local adaptation only in MN, where populations sampled from nearest to the garden tended to have the highest fitness (Fig. 3a,b). At IA, there was a weak relationship between fitness and distance from the garden. At the two southern gardens, IL and MO, the fitness of more southerly populations was higher than the fitness of populations sampled from near and north of the garden. Relative fitness of source populations was positively correlated among three of the gardens, IA, IL, and MO (Fig. 4). By contrast, population mean fitness at IL and MO was negatively, albeit weakly, correlated with fitness at MN.

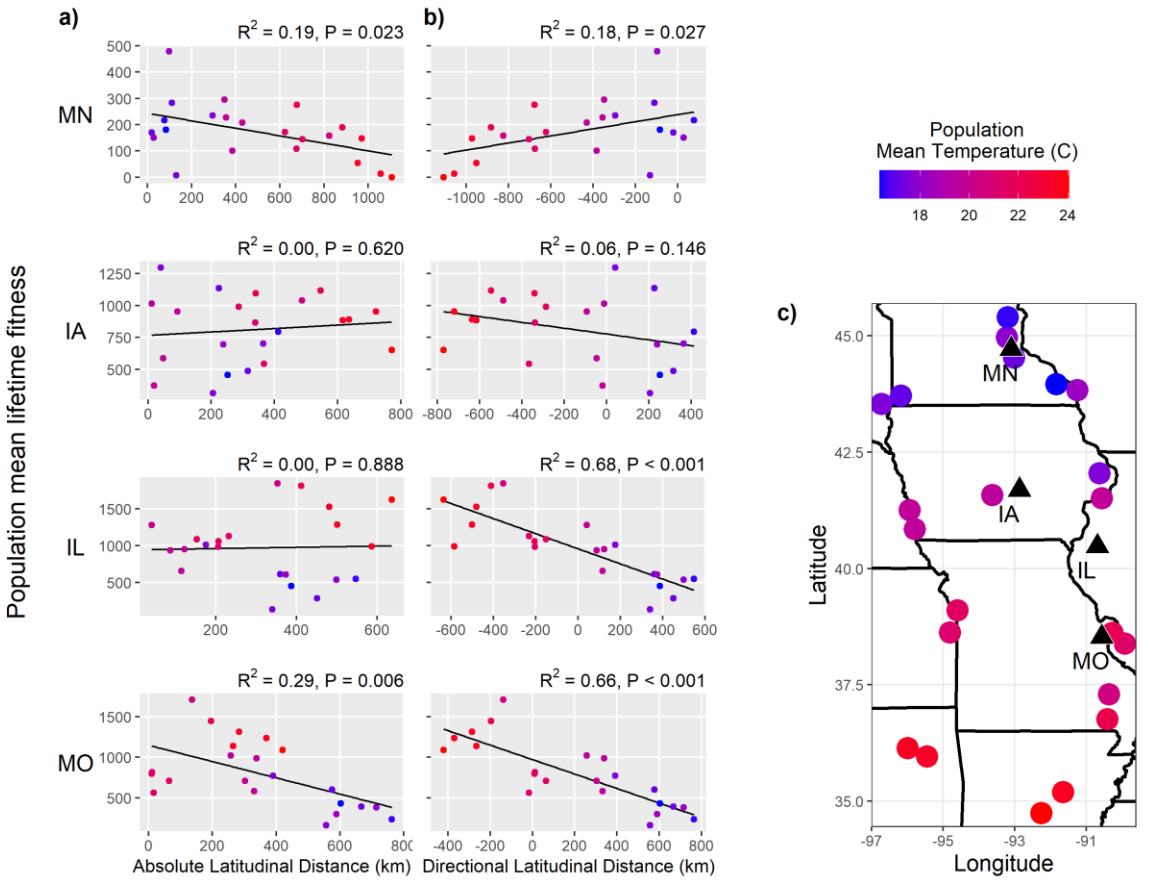


Figure 3. Linear regressions of mean lifetime fitness (number of fruits) on **a)** absolute and **b)** directional latitudinal distance between source populations and gardens. Each point represents mean fitness of a single population and is colored by population growing season mean temperature (averaged 1986-2015). Negative directional latitudinal distance indicates a population sourced from a latitude south of the garden. Latitudinal distance was transformed to distance in kilometers based on 1° ≈ 111 km. Garden and population locations **(c)**, with populations colored by population growing season mean temperature (averaged 1986-2015).

Predictors of fitness

We compared the relative explanatory power of absolute geographic distance, directional geographic distance, and climatic distance for explaining fitness variation among populations at each common garden. Both geographic distance metrics comprised longitudinal and latitudinal distance, but because longitudinal distance contributed little explanatory power (Table S5), we focus on regressions of *fitness ~ latitudinal distance*. Similarly, precipitation distance contributed very little explanatory power to our climatic models (Table S5). Temperature distance explained large amounts of variation in fitness at the gardens, but because temperature and directional latitudinal distance were tightly correlated (r = -0.95; Table S4), we discuss results in light of latitudinal distances below but include temperature information in figures and text to aid in interpretation (full climatic distance results can be found in Table S5).

Averaged across gardens, directional latitudinal distance was a much better predictor of population mean fitness than absolute latitudinal distance (mean R^2 = 0.40 and 0.12, respectively; Fig. 3; Table S5). However, this pattern differed among the four common gardens and was largely driven by the two southern gardens, IL and MO. Absolute and directional latitudinal distance explained similar amounts of fitness variation at MN, (R^2 = 0.19 and 0.18, respectively; although since MN is near the northern edge of the population sampling area, absolute and directional latitudinal distance were almost perfectly correlated, Table S4). Neither distance metric explained an appreciable proportion of fitness variation at IA (both $R^2 < 0.07$, $P > 0.1$). At the two southernmost gardens, IL and MO, population mean fitness was much better predicted by directional latitudinal distance than by absolute latitudinal distance. The importance of using directional distance was particularly apparent at IL; whereas absolute latitudinal distance explained effectively no fitness variation among populations at IL (adjusted R^2 = 0.00),

directional latitudinal distance predicted fitness variation very well ($R^2 = 0.68$). At both IL and MO, there was a strong trend of populations originating from warmer and more southerly regions than the gardens having the highest mean fitness.

Spatial scale of adaptation

We quantified the spatial scale of adaptation at each garden as the slope of the linear regression of population mean fitness on directional latitudinal distance (Fig. 3b). This slope presented below thus represents the predicted change in fitness with distance at each common garden. Slopes were generally shallow, indicating a large spatial scale of adaptation (mean absolute slope averaged across gardens = 57 fruits per 100 km). However, there was substantial variation in slopes among gardens, (slope ± SE: MN = - 13.5 ± 6; IA = 22.8 ± 15; IL = 102.5 ± 15; MO = 89.3 ± 14 fruits per 100 km). The two southern gardens, IL and MO, had steeper slopes, and thus a smaller spatial scale of adaptation, than the northern garden MN (Figs. 3b). These relationships remained, albeit of smaller magnitude, when slopes were scaled by garden mean fitness; the absolute values of garden relativized slopes in IL and MO (both ≈ 0.11) were about 40% larger than the slope in MN (0.08).

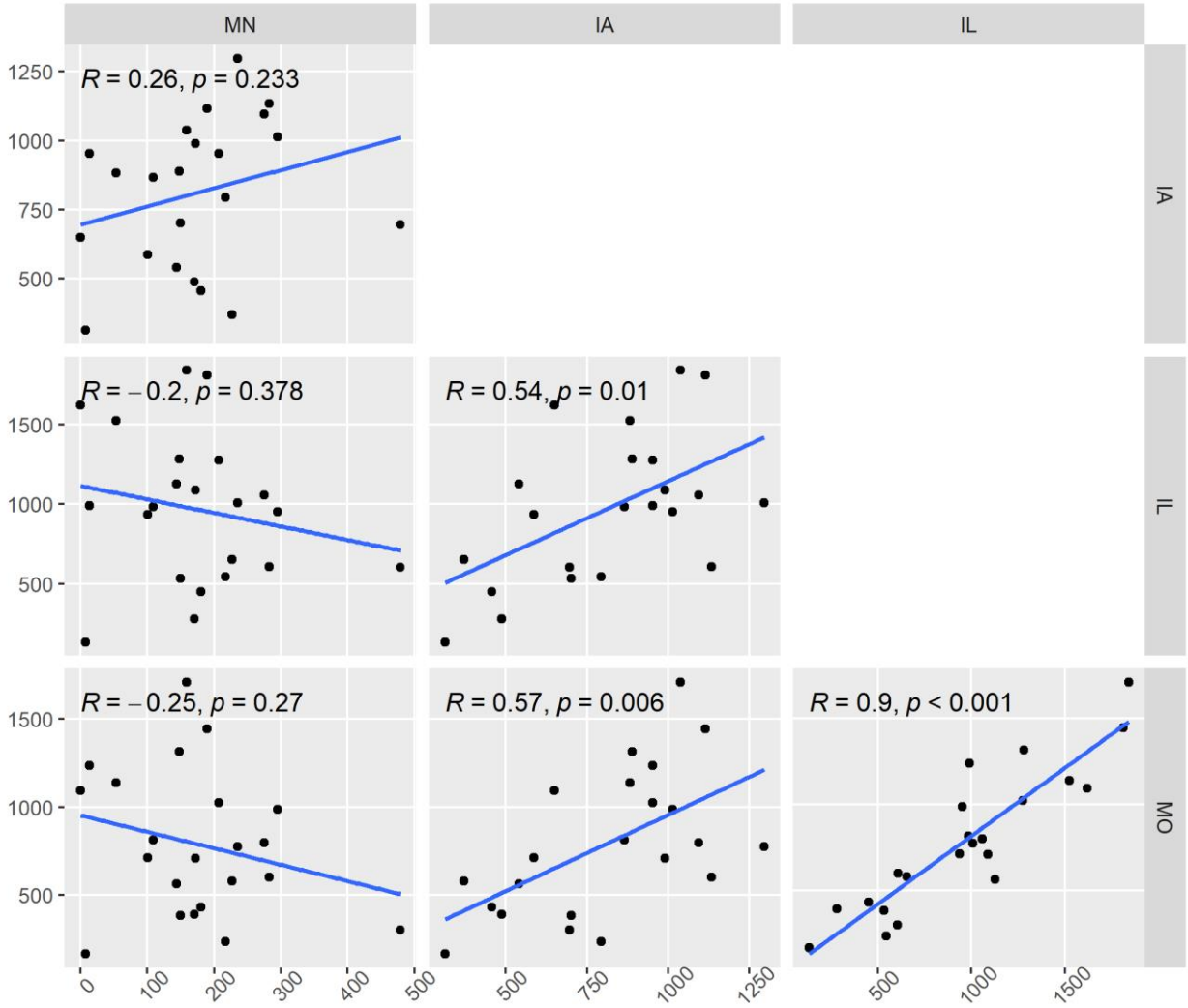


Figure 4. Correlation matrix of population mean fitness at the four common gardens. Pearson correlation coefficients are shown; Spearman rank coefficients are similar and listed in Table S6.

Variation across life history stages

To identify the life history stages that were most affected by the environment in which plants were grown, we partitioned lifetime fitness into three stages: probability of surviving to four weeks post-sowing, probability of fruiting given that a plant survived, and number of seeds produced given that a plant fruited. We found that early plant survival was high for all populations and all gardens and thus contributed little to among-garden differences in plant fitness or among-population fitness variation (Fig. 5). Similarly, at the three southernmost gardens, nearly all plants that survived fruited. By contrast, there was a strong relationship between latitudinal distance and probability of fruiting in the MN garden, where plants from more southern latitudes were much less likely to produce mature fruits before the end of the growing season (Fig. 5). This pattern most likely reflected maladaptation in flowering cues based on photoperiod; southern populations generally had delayed phenology relative to northern populations (Fig. S4). The strongest overall contributor to among-population fitness differences was number of fruits produced – at all four gardens, populations sampled from more southerly locations tended to produce more fruits than populations sampled from more northerly locations, although this relationship was not strong in IA.

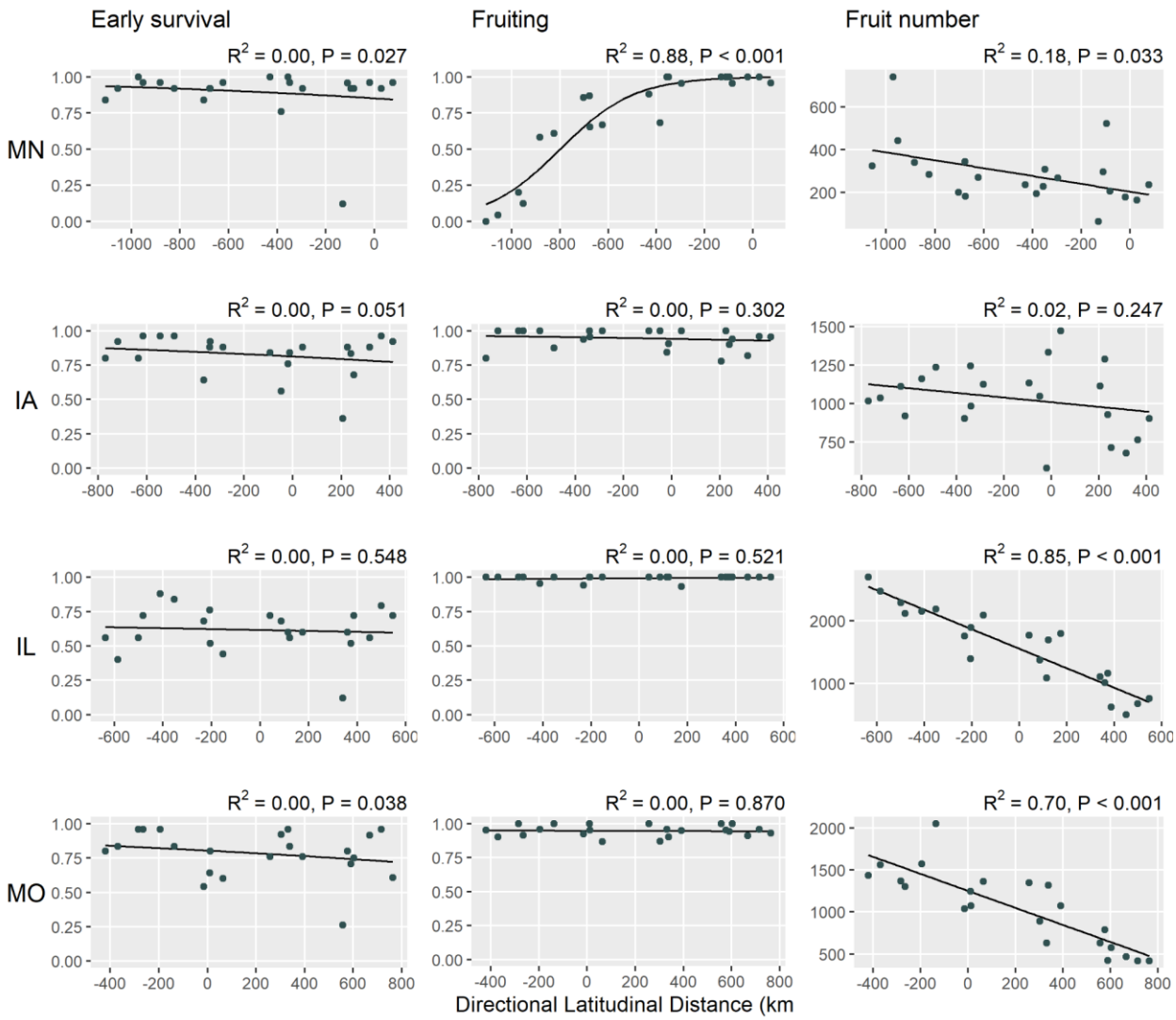


Figure 5. Relationships between conditional fitness components [*P*(early survival), *P*(fruiting), and number of fruits] on latitudinal distance for each common garden. Early survival and fruiting were modeled with logistic regression; fruit number with linear regression. ANOVA tables are in Table S7. Points represent population means. For logistic regressions (Early survival and Fruiting), the pseudo-R^2 is based on differences between measured population proportions and model-predicted probabilities. Early survival was measured for all plants; fruiting and number of fruits were predicted from phenotypic models for the subset of plants where these traits were not measured (SI "Predicting fitness"). Latitudinal distance was transformed to distance in kilometers based on $1° \approx 111$ km.

Discussion

The study of local adaptation provides insight into the spatial scale of adaptive differentiation, the influence of selection versus neutral processes on evolution, and the environmental drivers of population differentiation (Savolainen et al. 2007; Whitlock 2015; Briscoe Runquist et al. 2020). Understanding the spatial scale of adaptation is also central to predicting how populations will respond to a changing climate (Aitken et al. 2008; Anderson 2015). To understand spatial patterns of adaptation to climate in common ragweed, we performed a reciprocal transplant experiment at four common gardens with 22 populations sampled across a large portion of ragweed's geographic range in the central United States. Our results revealed three major insights: 1) there was evidence for local adaptation at the northern edge of the range, but not at the other gardens, 2) the overall spatial scale of adaptation to climate in ragweed is large, but southern gardens exhibited smaller scales of adaptation, and 3) climate change will likely increasingly disrupt patterns of local adaptation in ragweed, as evidenced by southern populations generally thriving at locations north of their home environment during the relatively warm experiment year.

Spatial scale of adaptation

The spatial scale of adaptation should reflect a balance between local selection pressures driving adaptive divergence and gene flow homogenizing populations (Slatkin 1987; Richardson et al. 2014). Although many transplant experiments have been conducted, only a few have provided a quantitative assessment of the spatial scale of adaptation (Antonovics and Bradshaw 1970; Galloway and Fenster 2000; Sexton et al. 2013; Anderson et al. 2015; Wright et al. 2018). We were interested in quantifying adaptation across a broad climatic gradient to examine adaptive

responses to historical climate and inform predictions of responses to climate change. Across the gradient sampled in our experiment, adaptation in ragweed occurs at a spatial scale of hundreds of kilometers. In IL, the garden with the steepest relationship between fitness and distance and thus the smallest spatial scale of adaptation, our model predicted fitness to decrease by about one fruit per km; thus it would take ~800 km for fitness to decline 50% from its predicted maximum (Fig. 3b). Adaptation at such a large spatial scale likely reflects both the high rates of gene flow among native populations of ragweed (Hämälä et al. 2020), which tend to be highly fecund and produce large amounts of wind-dispersed pollen, and the fact that the main agents of selection captured by our sampling design, climate and daylength, vary at relatively large scales.

While the overall spatial scale of adaptation revealed in our experiment was large, there was variation among gardens in this measured scale. In the northern garden, MN, fitness declined gradually with distance. By contrast, in the two southern gardens (IL and MO) the relationship between fitness and distance to the source population was substantially steeper. The biology underlying this difference is illuminated by examining how the life-history stages responsible for fitness variation differed among gardens. In MN, plants from southern populations were much less likely to fruit than plants from more northern populations, presumably due to adaptive differences to phenological cues. Nevertheless, in MN the fecundity of southern plants that did fruit was generally much larger than the fecundity of plants from northern populations, which made the relationship between fitness and distance more shallow. By contrast, in the southern gardens IL and MO, there were few among-population differences in survival or probability of fruiting. Rather, the higher fitness of more southern populations was driven almost entirely by those populations having much higher fecundity than northern populations, resulting in a steeper relationship between fitness and distance.

Climate change

These patterns of adaptation have important implications for ragweed's response to climate change and developing predictions about its shifting range dynamics with further warming. In the southern gardens we observed a pattern of disrupted local adaptation due to warm temperatures, where the most fit populations were those sourced from locations warmer than the garden (Figure 3b). This mirrors the expectation shown in Figure 1b, where peak fitness is shifted away from zero (local populations) and toward warmer populations. Similar signatures of climate change were revealed in a recent meta-analysis, where population home-site advantage decays with increasing differences between contemporary and historical temperatures (Bontrager et al. 2020). Despite the spatial scale of adaptation in ragweed being large, our results indicate that the sheer magnitude of climate change will still lead to local maladaptation of many ragweed populations. For example, the temperature in our gardens was approximately one degree warmer than historical averages, which is roughly equivalent to moving 165 km south along the latitudinal gradient from which we sampled. However, our experiments suggest that the magnitude of maladaptation will not be consistent across the species' range. The disrupted local adaptation we found was driven by the two southern gardens where adaptation to climate was stronger. In contrast, we found no evidence of local maladaptation in the northern garden, MN, where we suspect phenological cues (especially daylength) are important determinants of fitness. These differing responses to similar climatic deviations suggest southern populations may be more affected by warming temperatures than northern populations, which experienced less variation in fitness across gardens and outperformed foreign populations in their home garden despite above average temperatures.

Though populations will certainly experience environmental changes *in situ* for some period of time, a common prediction is that as climate warms, populations may shift northward and track the climatic envelope to which they are currently adapted, a prediction that has already borne out for some species (Chen et al. 2011; Rumpf et al. 2018). Our results from the two southern gardens suggest that individuals from southern populations that migrate northward will likely do well across much of the range and may outperform local populations. However, at the northern edge of the range, growing season length may lengthen with climate change, but daylength will not, and the fitness of southern populations migrating northwards will largely depend on adaptive evolution of flowering cues (Griffith and Watson 2006). Taken together, our results suggest that in response to a changing climate, more southerly populations of ragweed might shift northward, or gene flow from southerly populations may accelerate adaptation of central populations to warmer conditions, potentially resulting in greater fecundity and larger populations. By contrast, without adaptation in phenological cues, daylength at the northern edge of the range may act as a barrier to range expansion. Thus, climate change could conceivably lead to a contraction of ragweed's geographic range from the south, as southern populations migrate northward, but migration at the northern edge is prevented or slowed, by constraints on adaptation to photoperiod. This highlights the important observation that the environment, and thus adaptation, is multivariate (Antonovics 1976; Benning and Moeller 2019), and populations' responses to rising temperature, either via migration or adaptation, will interact with other important axes of adaptation and will likely differ from predictions based solely on climatic change.

Methodological considerations

Our results emphasize two critical methodological considerations when attempting to understand fitness variation as a function of distance between populations and common gardens. First, the garden-dependent patterns of adaptation we detected point to the importance of environmental context, i.e., G×E, on the assessment of local adaptation (Des Marais et al. 2013; Halbritter et al. 2015; Lowry et al. 2019; Runquist et al. 2020). If we had conducted our experiment only in MN, we would have concluded that common ragweed is adapted to the local environment. If we had conducted it only in IA, we would have concluded that there is no geographic pattern in adaptation. If the experiment was restricted to IL or MO, we would have concluded that populations have strongly diverged across latitude but that populations are maladapted to current climatic conditions. These results highlight how employing multiple common gardens across a large portion of a species' range is essential in experiments seeking to understand patterns of local adaptation in a species and responses to a changing climate. The inclusion of many source populations is also key in such studies, as it enables one to treat distance as a continuous, as opposed to categorical (e.g., home vs. away) predictor in analyses, resulting in an easily interpretable estimate of the spatial scale of adaptation as the change in fitness per unit distance.

A second methodological consideration relates to how "distance" is measured when assessing spatial patterns in adaptation among populations. We found that directional latitudinal distance was generally a far better predictor of lifetime fitness than absolute latitudinal (or absolute Euclidean) distance. There are two main advantages of including directional geographic distance in analyses characterizing spatial patterns of adaptation. First, directional geographic distance, unlike naive geographic distance, better captures variation due to environmental factors that are spatially autocorrelated (such as latitude here, which captures a gradient in both

temperature and photoperiod). Second, directional geographic distance is easy to interpret, especially when contrasted with principal component approaches often used to reduce dimensionality of environmental variation. As opposed to quantifying environmental distance in principal components space, one can directly quantify patterns of local adaptation as the expected change in fitness per kilometer. This metric is of much greater potential use to land managers and restoration biologists faced with the problem of conservation under climate change than one based on abstracted multivariate environmental distances.

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Supplementary Information for**:**

The spatial scale of adaptation in a native annual plant and its implications for responses to climate change

Predicting fitness

Measuring female fitness on all plants was not possible in the field due to logistical constraints. However, we did collect extensive phenotypic data preceding fruit set, from planting until 16 weeks post planting, for all plants. Thus, we used the full lifetime fitness data we had from the subset of 9 populations to build models based on individual plant phenotypes that predicted the two final fitness components of interest: probability of producing fruit, and number of fruits. We used these models to predict P(fruiting) and number of fruits for the remaining plants in the data set (those from the other 13 populations, and individuals from the 9 populations whose fitness was not estimated in the field). Final data set sizes were N = 524 for the model building data set (hereafter, modeling set) and N = 1130 for the prediction data set (hereafter, prediction set). These datasets excluded any plants that we know died (N = 530), because their fitness was known to be zero.

We used logistic regression to model P(fruiting) as predicted by garden, plant stage at 12 weeks, and their interaction, with model formulation *glm(fruiting ~ Garden X Stage_12wk, family = binomial)*. This model correctly predicted fruiting in 508/524 individuals (97%), after rounding estimated probabilities to 0 or 1. We then used this model to predict P(fruiting) for all individuals in the prediction set, rounding this probability to 0 or 1.

We used linear regression to model fruit number as predicted by garden, height at 16 weeks, stage at 12 weeks, and their interactions, and the interaction of garden and female

flowering stage at 12 weeks; the model took the form *lm(fruits ~ Garden X Height_16wk X Stage_12wk + Garden:Female_12wk)*. Model $R^2 = 0.38$. Though the model had a modest R^2, what we ultimately were interested in was whether these individual level phenotypic models could accurately predict mean population values, as this was the unit of analysis in our main analyses. When we calculated predicted population mean fruit set based on these individual plant predictions, the correlation between observed and predicted population mean fruit set was very high, $r = 0.94$. Examining the relationship between one of our distance metrics, directional latitudinal distance, and the observed vs. predicted mean population fruit set, we can see the data sets produce extremely similar regression results (Fig. S1). We used this linear model to predict the number of fruits produced for all individuals in the prediction set. After predicting P(fruting) and number of fruits for all individuals in the prediction set, we calculated fitness for those individuals as the product of those two numbers. These predictions produced one outlier at MN, whose predicted fitness, due to the plant's extremely large size, was almost four times that of most fecund individual measured in that garden (though similar to some individuals measured at other gardens). We kept this outlier in the data set because it had no qualitative effect on our analyses (regression of *fitness ~ directional latitudinal distance* at MN garden: with outlier, slope = 0.135 fruits per km, $P_{1,20} = 0.027$, $R^2 = 0.18$; without outlier, slope = 0.10 fruits per km, $P_{1,20} = 0.038$, $R^2 = 0.16$.)

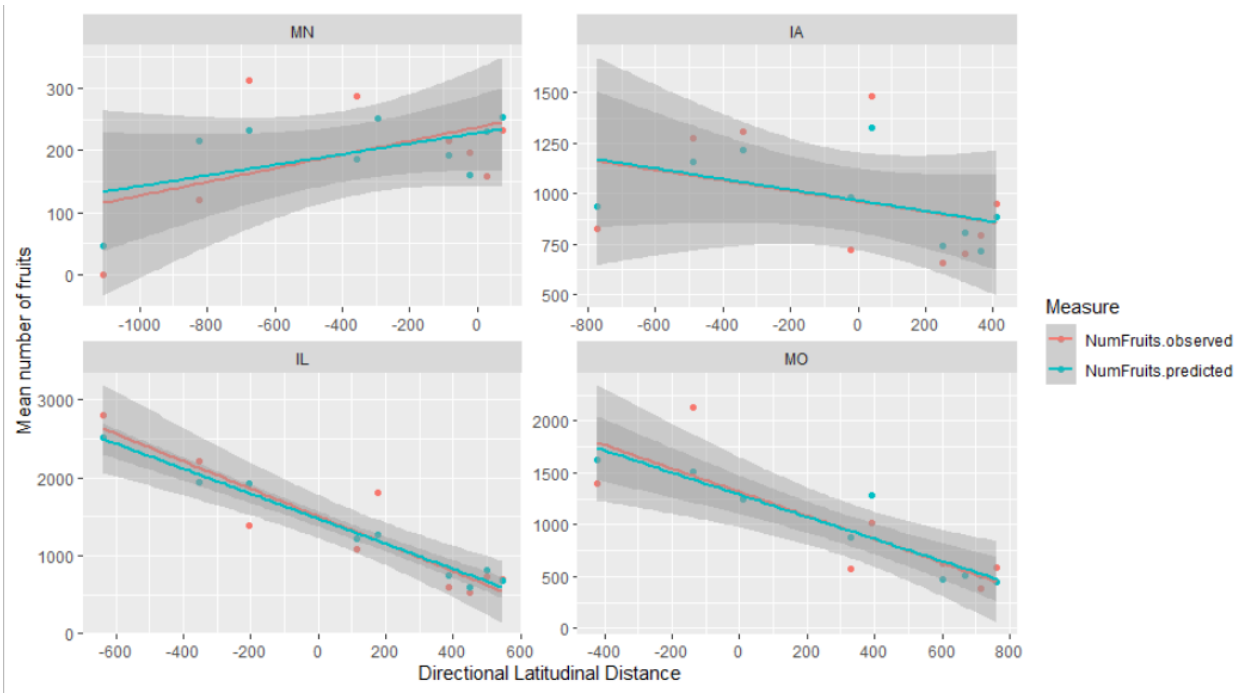

Figure S1. Population mean number of fruits regressed on directional latitudinal distance at each garden, for surviving individuals in the nine "focal" populations. Red points and lines are observed values and the associated linear regression; teal points and lines are predicted values and linear regression.

Of course, the important assumption in this approach is that the modeled relationship between earlier phenotypic data and fruit set in the 9 "focal" populations for which we had fruit set data holds for individuals from the 13 "extra" populations for which we did not record final fitness data. As a proof of concept, we took a similar approach to the one we used for fruit set to model the probability of a plant having any mature fruits at 16 weeks, which was phenotypic data we recorded for every surviving plant in the field and the latest life history data we had for every plant. We modeled the probability of a plant having any mature fruits at 16 weeks as predicted by garden, female flowering stage at 12 weeks, height at 12 weeks, and their interactions, with logistic regression, with model formulation *glm(fruiting_16wk ~ Garden X FemaleStage_12wk X Height_12wk, family = binomial)*. We built this model using individuals

from the nine focal populations, and then used it to predict the probability of fruiting at 16 weeks for individuals in the 13 extra populations, rounding probabilities to 0 or 1. These predictions matched the observed data in the 13 extra populations very well (Fig. S2). Overall in the 13 extra populations, we observed 18.2% of surviving plants had mature fruits at 16 weeks; model predictions resulted in an estimate of 16%. A two-proportions Z-test between the predicted and observed data indicate no significant difference (P = 0.22)

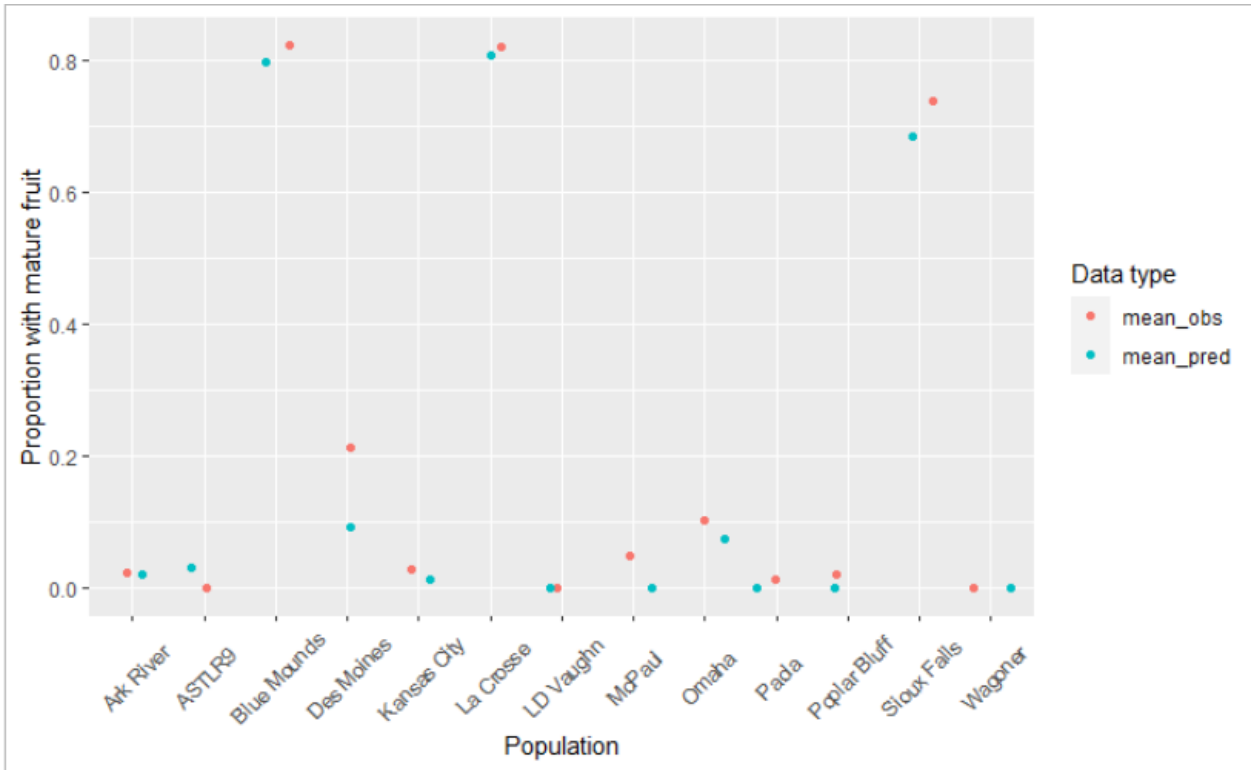

Figure S2. Proportion of surviving plants with mature fruit at 16 weeks for the 13 "extra" populations, based on observed data (red points) and values predicted from a model built using the 9 "focal" populations (teal points).

We can also ask whether the relationship between fitness and distance seems to change between the two sets of populations, or whether using the full data set with predictions changes our inference relative to the data set with only the nine populations for which fruit set was measured in the field. In Figure S3, we can see that regardless of the data set used (9 "focal"

populations, 13 “extra” populations, or all 22 populations combined), the relationship between distance and fitness remains similar.

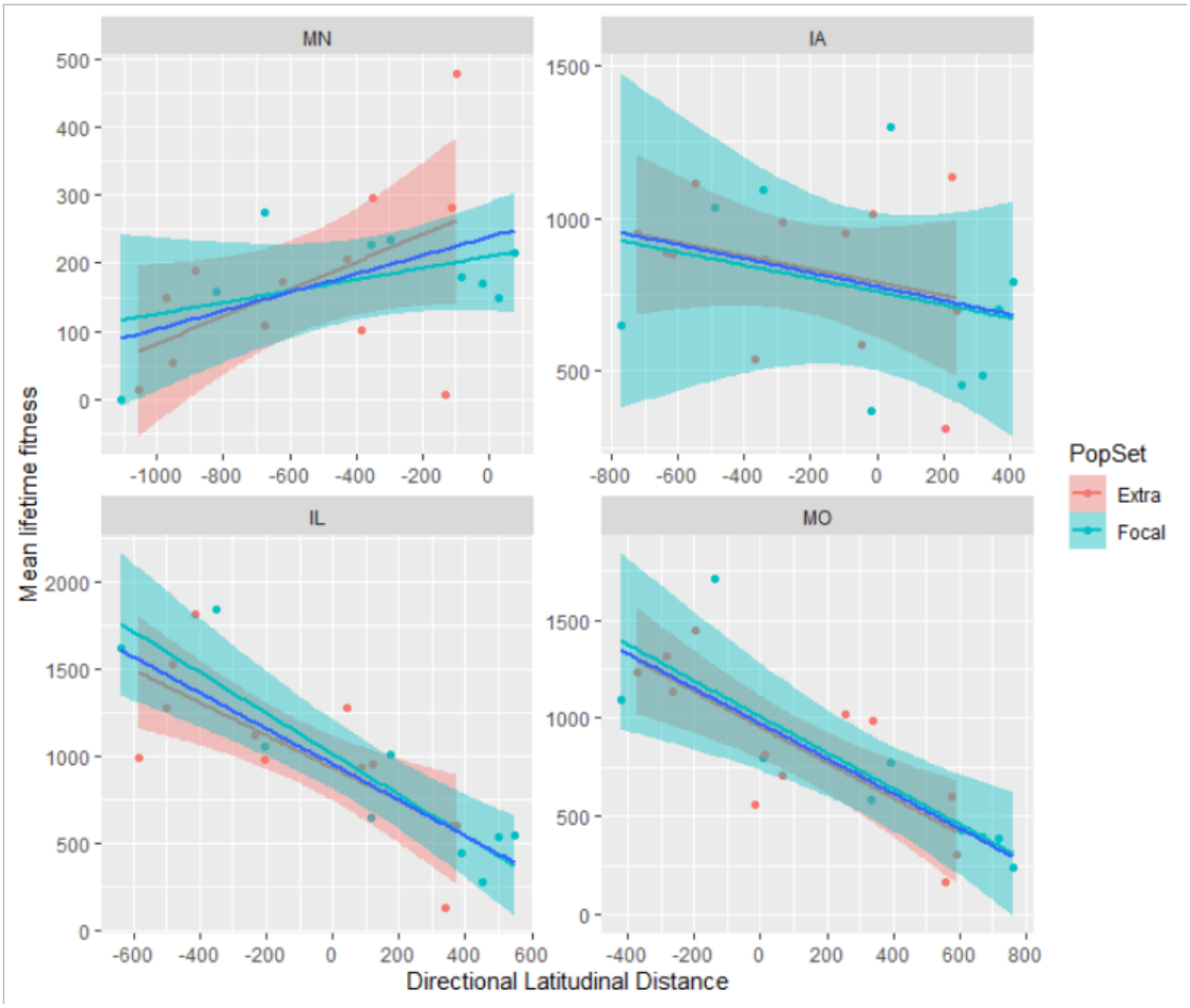

Figure S3. Mean lifetime fitness regressed on directional latitudinal distance. Teal points and linear regression lines/bands are based on the “focal” 9 populations only; red points and linear regression lines/bands are based on the “extra” 13 populations only; the blue linear regression (no band) is based on the full data set with 22 populations.

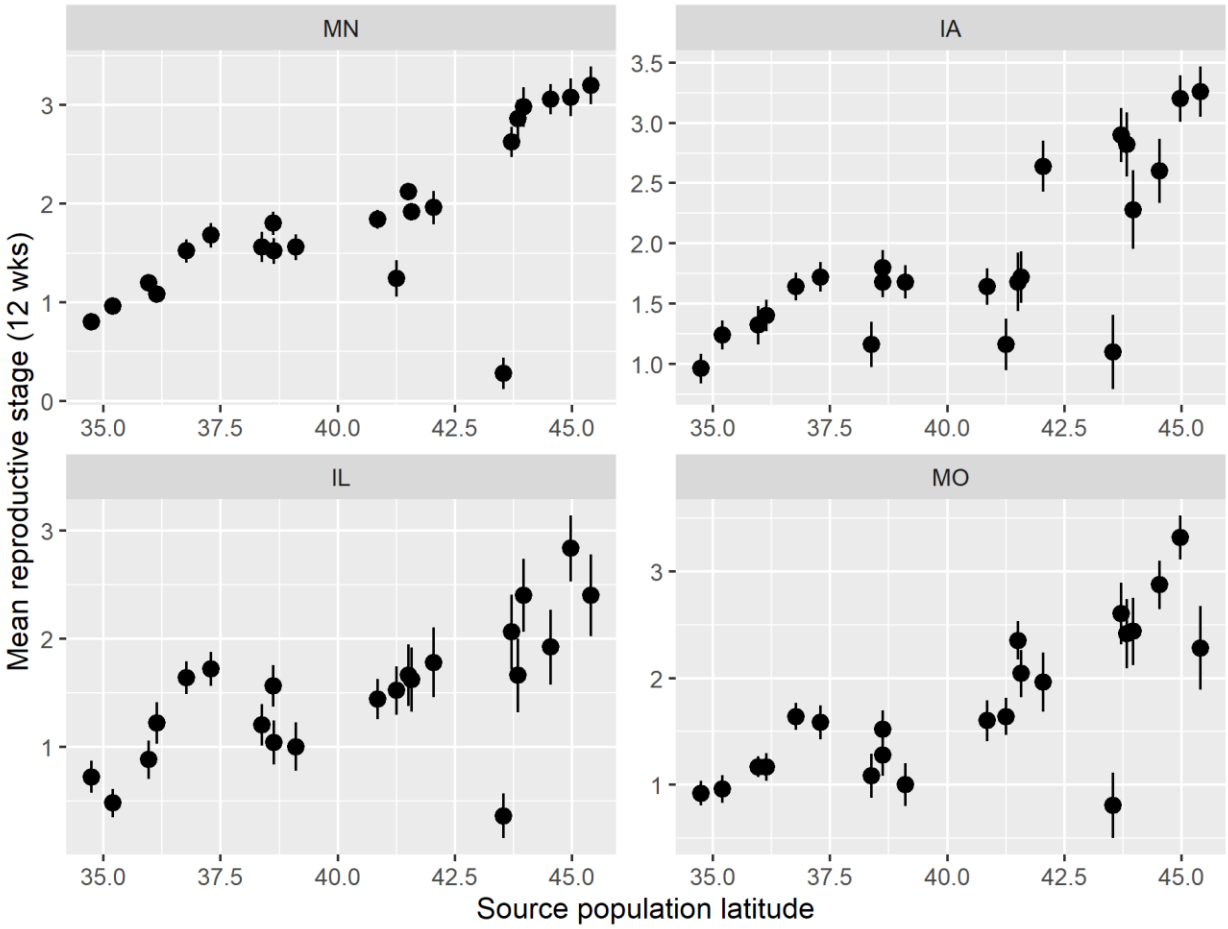

Figure S4. The effect of source population latitude on overall plant reproductive stage at 12 weeks (mean ± SE) at each common garden. Values above 3 indicate either male or female flowers were open. See Table S3 for a description of all potential stages.

SI Tables

Table S1. Description of populations included in the latitudinal reciprocal transplant experiment. Populations are listed in ascending order by latitude. *n* indicates the number of maternal plants that were included from that population. Populations that had a fruit count in the field are indicated with a "Y".

Population	Latitude	Longitude	*n*	Fruit counts
LTR	34.74136566	-92.25947634	23	Y
LDV	35.19796198	-91.64370698	25	
WAG	35.96535395	-95.45782392	25	
ARK	36.13697554	-95.99441688	19	
POP	36.76623098	-90.40547492	15	
CLW	37.29567794	-90.36880106	25	Y
SR9	38.38645	-89.92686	25	
SU8	38.61736	-90.26415	25	Y
PAO	38.62834563	-94.81757954	25	
KAC	39.10268604	-94.60068525	25	
MPL	40.84571607	-95.80212162	25	
OMA	41.25688838	-95.94715796	12	
ROI	41.51026297	-90.56311849	23	Y
DMS	41.57788835	-93.62835764	25	
PRC	42.05130841	-90.63232642	25	Y
SFA	43.53882148	-96.72653811	24	
BMO	43.71449138	-96.18077593	25	
LAC	43.83766655	-91.2513499	25	Y
LEW	43.96234593	-91.83442563	18	
R10	44.53656	-93.02021	25	Y
U25	44.96804	-93.22023	18	Y
R16	45.40264	-93.19718	25	Y

Table S2. Location of common gardens

Common garden	Latitude	Longitude	Hosting Institution
MN	44.71645	-93.09789	Rosemount Outreach and Research Center, University of Minnesota
IA	41.68763	-92.86527	Conrad Environmental Research Area, Grinnell
IL	40.47617	-90.6844	Western Illinois Agricultural Station, Western Illinoi University
MO	38.53701	-90.5589	Tyson Research Center, Washington University

Table S3. Vegetative and reproductive scoring that was used on each individual plant at weeks 4,8,12 and 16. Note that an individual could receive an intermediate score between stages.

Stage	Overall plant	Male flowering	Female flowering
1	Vegetative; only leaves	Male inflorescences developing, only buds visible	Few stigmas visible
2	Initiation of reproduction	Male inflorescences visible, but flowers closed	Many stigmas visible, few immature fruit
3	Male or female flowers open	<50% flowers open per inflorescence	Many immature fruit visible, few stigmas
4	Fruit visible	>50% flowers open per inflorescence	Many immature fruit visible, few mature fruit
5	Senesced	All male flowers open or flowers senesced	Many mature fruit or senesced

Table S4. Pearson correlation (*r*) among distance metrics used in the analyses, for all gardens together (top, bolded) and individual gardens.

	Absolute Latitudinal Distance	**Directional Latitudinal Distance**	**Directional Temperature Distance**
Directional Latitudinal Distance			**-0.95** MN (-0.96) IA (-0.96) IL (-0.96) MO (-0.96)
Absolute Latitudinal Distance		**-0.41** MN (-1) IA (-0.67) IL (-0.18) MO (0.7)	**0.35** MN (0.96) IA (0.65) IL (0.16) MO (-0.68)
Absolute Geographic Distance	**0.93** MN (0.99) IA (0.96) IL (0.72) MO (0.86)	**-0.37** MN (-0.99) IA (-0.74) IL (-0.13) MO (0.64)	**0.32** MN (0.95) IA (0.71) IL (0.15) MO (-0.59)

Table S5. ANOVA (Type II sums of squares) tables for linear regression models at each garden, with separate models for straight-line absolute geographic, absolute geographic (decomposed into latitude and longitude) directional geographic (latitude and longitude), and climatic (temperature and precipitation) distances as predictors.

			MN			**IA**			**IL**			**MO**		
	Term	Df	Sum Sq	*F*	*P*	Sum Sq	*F*	*P*	Sum Sq	*F*	*P*	Sum Sq	*F*	*P*
Straight-line geographic	*Distance (km)*	1	59409	6.25	0.021	16474	0.22	0.647	359	0.00	0.969	1113227	8.96	0.007
	Residuals	20	190194	**R^2 = 0.20**		1525874	**R^2 = 0.00**		4611538	**R^2 = 0.00**		2485503	**R^2 = 0.27**	
Absolute geographic	*Latitude*	1	57857	5.76	0.027	27328	0.35	0.563	871	0.00	0.952	1105756	8.66	0.008
	Longitude	1	1092	0.11	0.745	26312	0.33	0.570	136977	0.58	0.455	22828	0.18	0.677
	Residuals	19	190796	**R^2 = 0.16**		1496757	**R^2 = 0.00**		4470227	**R^2 = 0.00**		2426965	**R^2 = 0.25**	
Directional geographic	*Latitude*	1	66422	7.77	0.012	149978	2.06	0.167	3020426	41.61	<0.001	2350951	37.97	<0.001
	Longitude	1	31867	3.73	0.069	2252	0.03	0.862	45637	0.63	0.438	2532	0.04	0.842
	Residuals	19	162462	**R^2 = 0.28**		1381859	**R^2 = 0.01**		1379168	**R^2 = 0.67**		1176495	**R^2 = 0.64**	
Climatic	*Temperature*	1	56373	5.55	0.029	46699	0.63	0.438	2031666	20.12	<0.001	1479508	16.14	0.001
	Precipitation	1	11768	1.16	0.295	30968	0.42	0.526	24147	0.24	0.630	3619	0.04	0.845
	Residuals	19	193088	**R^2 = 0.15**		1411246	**R^2 = 0.00**		1918626	**R^2 = 0.54**		1741442	**R^2 = 0.47**	

Table S6. Pearson (top) and Spearman rank (bottom) correlation matrix for population mean fitness among gardens.

	MN	**IA**	**IL**
IA	0.265 0.417		
IL	-0.198 -0.186	0.537 0.523	
MO	-0.246 -0.202	0.566 0.579	0.896 0.859

Table S7. ANOVA tables for life history stage analyses at each common garden. Directional latitudienal distance was the only predictor in each model. Early survival and fruiting were modeled with logistic regression; fruit number was modeled with simple linear regression.

	***P*(Early survival)**		***P*(Fruiting)**		**Fruit number**	
	χ^2	*P*	χ^2	P	*F*	*P*
MN	4.86	0.027	252.69	< 0.001	5.26	0.033
IA	3.82	0.051	1.07	0.302	1.42	0.247
IL	0.36	0.548	0.41	0.521	118.38	< 0.001
MO	4.31	0.038	0.03	0.870	50.70	< 0.001