RESEARCH ARTICLE

Common milkweed seeds exhibit latitudinal clines more consistent with adaptation to growing season length than temperature

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Overwintering monarch (*Danaus plexippus*) populations have declined since the 1990s. In response, restoration of milkweeds, including *Asclepias syriaca* (common milkweed), an important host plant in their breeding grounds, has become increasingly common. However, latitudinal variation in milkweed populations suggests the possibility of regional adaptation and the potential for seed provenance to affect restoration success. Using seeds from 20 populations throughout the range of *A. syriaca*, we tested whether seed mass, germination success, and germination time in the greenhouse demonstrate geographic clines consistent with available evidence for this species from other studies. In addition, we tested for patterns in germination traits consistent with adaptation to spring thermal conditions by planting seeds from 10 populations in growth chambers simulating Minnesota and Kentucky spring temperatures. Even after accounting for seed mass, seeds from higher latitudes germinated faster on average under all conditions. Elevated temperatures accelerated germination time and leaf development time; however, we did not detect geographic patterns in leaf development time, indicating that the processes underlying the latitudinal cline in germination time may be unique to the germination stage. In the thermal adaptation study, high-latitude populations produced larger seeds and seeds that germinated at a higher rate; however, neither latitudinal trend was observed in the geographic clines study, even though individual seed mass predicted germination success. High-latitude populations express more favorable germination traits in every setting measured, perhaps due to reduced dormancy. Consequently, we conclude that latitudinal clines are more consistent with adaptation to growing season length than to spring temperatures.

Key words: *Asclepias syriaca*, geographic cline, germination traits, intraspecific variation, population differentiation, seed dormancy, thermal time

Implications for Practice

- Given that some milkweed germination traits show consistent latitudinal clines, we provisionally recommend sourcing seeds from similar ecoregions or climates.
- Although seeds from high-latitude populations germinate quicker, which could be favorable for restoration, more data about fitness across life stages are necessary to evaluate seed sourcing strategies that deviate from climate-matching.
- Seeds from lower latitudes appear more dormant and may require longer periods of cold stratification prior to sowing in the spring.
- Although 4 weeks of cold stratification is commonly recommended for common milkweed seeds, a full year of cold stratification was not harmful for most accessions and appears beneficial, especially for low-latitude seeds.
- Larger seeds have germination traits that are likely to improve establishment.

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Introduction

Asclepias syriaca (common milkweed) has declined by 50–90% in the United States, with estimates varying with geographic

area, time frame, habitat, and method of study (Pleasants & Oberhauser 2012; Zaya et al. 2017; Boyle et al. 2019). Milkweed species are the only host plants for *Danaus plexippus* (monarch butterfly) larvae in North America, so declines in milkweed may contribute to declining monarch populations (Pleasants & Oberhauser 2012; Stenoien et al. 2018). Consequently, some advocate for widespread planting of milkweeds (Thogmartin et al. 2017). Common milkweed in particular is an important food source for migrating monarchs (Seiber et al. 1986; Malcolm et al. 1993), so restoration projects often focus on increasing its abundance (Thogmartin et al. 2017).

Restoration practitioners must decide how to source seeds for restoration. Because local adaptation is widespread (Hereford 2009; Leimu & Fischer 2008), introducing seeds from different environments may result in reduced restoration success. For example, seeds in novel environments may miss germination cues, mistimed emergence could cause seedling death, and gene flow can cause outbreeding depression or long-term collapse (Kramer & Havens 2009). Consequently, generalized seed transfer zones have been developed to support selecting seeds that come from similar environments (Hufford & Mazer 2003; Bower et al. 2014). However, seed availability and cost probably drive most decision-making, especially in the absence of data favoring a particular seed-sourcing strategy.

Alternatively, it may be advantageous to source seeds from diverse populations, including those that match projected future climates (Kramer & Havens 2009; Weeks et al. 2011; Breed et al. 2013). This practice may improve evolutionary potential under changing climate, but it risks disrupting local adaptation to non-climate-related factors (Aitken & Whitlock 2013). Thus, characterizing the relative importance of climate- and non-climate-related adaptations is important for restoration. Patterns of adaptation can vary across different life stages of the same species, and since most restoration projects use seeds, specific studies of adaptation at early life stages are important for restoration (Gibson et al. 2016).

Common garden studies provide evidence consistent with adaptive population differentiation when traits are associated with geography of origin—called a cline. Clines in germination traits can be idiosyncratic across species, precluding generalized recommendations for seed sourcing (Cochrane et al. 2015; Seglias et al. 2018). Thus, it is important to investigate environmental clines specific to *A. syriaca* to identify factors to consider when selecting seeds for restoration.

Geographic variation in *A. syriaca* traits, including growth, phenology, defense, seed mass, and germination rates have been documented in parts of the range (Malcolm et al. 1989; Woods et al. 2012; Finch et al. 2018); however, there is not strong evidence for adaptation to local conditions (Finch et al. 2023). *Asclepias syriaca* plants from higher latitudes produce larger seeds (Woods et al. 2012) with higher germination success (Finch et al. 2018) and faster germination times (Finch et al. 2023). These latitudinal clines are consistent with adaptation to temperature, growing season length (GSL), or some other aspect of the environment that varies with latitude (De Frenne et al. 2013).

The thermal time hypothesis predicts a tradeoff between the basal temperature (the minimum temperature at which
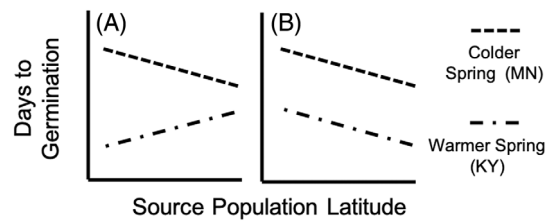

Figure 1. Alternative predictions for adaptation to temperature (A) or GSL (B). (A) Adaptation to temperature predicts crossing reaction norms, such that seeds originating from high latitudes germinate relatively faster under colder springs, such as in MN; whereas, seeds originating from lower latitudes germinate relatively faster under the warmer springs, such as in KY. (B) Adaptation to GSL predicts parallel reaction norms in which seeds from high latitudes germinate relatively faster under all thermal conditions.

development occurs) and the thermal time, measured in degree days, to complete a developmental process (Bonhomme 2000; Trudgill et al. 2005; Dürr et al. 2015). Specifically, individuals from colder latitudes are predicted to start development and germinate earlier in colder temperatures than those from warmer latitudes. In contrast, individuals from warmer latitudes have warmer basal temperatures but can develop faster, so they are predicted to germinate earlier in warmer environments (crossing reaction norms, Fig. 1A; Trudgill et al. 2005).

Alternatively, GSL also varies predictably with latitude, and could select for consistent clines if genotypes from environments with shorter growing seasons exhibit consistently faster development (Conover & Schultz 1995; Belk et al. 2005; Toftegaard et al. 2016) or consistently earlier phenologies (Fig. 1B). Indeed, common garden studies, including one with common milkweed (Woods et al. 2012), have found that higher latitude plants have earlier phenologies (Turesson 1930; Olsson & Ågren 2002; Zettlemoyer et al. 2017).

Applying the thermal time concept to milkweed seeds is challenging because germination requires two processes: dormancy breaking and germination. Milkweed seeds at maturity are conditionally dormant, meaning they can germinate in a specific period of time under a narrow range of conditions (Finch-Savage & Leubner-Metzger 2006). Dormancy decreases with time in dry storage (called after-ripening) and cold-moist stratification (Finch et al. 2018). As dormancy breaks, the range of environmental conditions under which germination occurs expands. Dormancy primarily affects germination success (Batlla & Benech-Arnold 2015), but dormancy-breaking processes such as cold stratification can also alter germination time, for example, by reducing the basal temperature necessary for germination (Steadman & Pritchard 2003). If dormancy is an adaptive trait, we might expect greater dormancy in plants experiencing colder winters to reduce risks of early germination (Meyer et al. 1995). However, Finch et al. (2018) reported greater germination success in milkweed seeds from higher latitudes, especially at colder temperatures and with less cold stratification, suggesting that seeds from northern populations are less dormant.

We aimed to characterize geographic clines (GCs) and test for adaptation to thermal spring conditions in common milkweed

seeds to support strategic restoration of milkweed. Given previous observations of latitudinal clines and the general importance of temperature for seed traits (Batlla & Benech-Arnold 2015), we hypothesized that temperature drives geographic patterns in seed and germination traits, implying that seeds for restoration should be sourced from environments similar to current or projected thermal conditions at a site. This hypothesis led to two predictions:

(1) Seed and germination traits in *A. syriaca* would show GCs with latitude of origin, since latitude is closely associated with temperature. We tested for clines in germination success and germination time by collecting seeds from 20 populations across the range (Lat: 36–47°N; Long: 71–96°W) and planting them in 2 different greenhouses.
(2) The development time of *A. syriaca* seeds would demonstrate patterns consistent with adaptation to thermal conditions, with seeds sourced from environments climatically similar to their growing temperature germinating faster than those from dissimilar environments (Fig. 1A). We tested for adaptation to thermal conditions by planting seeds from 10 populations in growth chambers simulating northern and southern thermal spring conditions.

Methods

Natural History

Asclepias syriaca is a weedy perennial native to the Great Plains and Northeast regions of the United States (Woodson 1954). Milkweed pollination occurs via transfer of pollinia, meaning seeds in each follicle are full siblings. Seeds are wind-dispersed and most are conditionally dormant at maturity; dormancy declines during after-ripening and especially after a period of cold-moist stratification (Baskin & Baskin 1977; Finch et al. 2018). No studies have identified persistent seed banks for milkweed species (Johnson & Anderson 1986; Csontos et al. 2009; Kaye et al. 2018).

Seed Collection and Maternal Plant Traits

Seeds were collected by volunteer educators participating in the Milkweed Adaptation Research and Education Network (marenweb.com) from 25 sites spanning the U.S. range of common milkweed (Table S1; Fig. 2). Mature, individual seed pods from naturally occurring *A. syriaca* plants were collected from five stems that were at least 5 m apart, air dried, and mailed to St. Olaf College. Seeds from each pod were counted, massed, and stored at ~3°C. Seeds that appeared to be unfilled were excluded. Most collections occurred during 2017 or 2018, but two additional sites were added in 2020 (Table S1). Volunteers submitted data on maternal plant height and number of seed pods for 16 source populations (indicated in Table S1), which we use to make inferences about possible maternal effects on seeds.

Source Environmental Data

If GCs in traits exist, they suggest adaptation to some environmental variable associated with geography. To make inferences about environmental characteristics associated with GCs, source populations were georeferenced using ArcMap (v. 10.5.0; ESRI 2011). Soil attributes (Soil Survey Staff 2005) and BioClim variables (Fick & Hijmans 2017) were extracted. Site-specific soil attributes represent weighted averages of the soil layers for cation exchange capacity (CEC) at pH 7 (a measure of how readily the soil holds nutrients), available water capacity (cm water/cm soil), percent soil organic matter, and reaction pH. Growing season length, was the expected number of frost-free days from 1961 to 1990 (Soil Survey Staff 2005). We limited the BioClim variables to mean annual temperature, temperature seasonality (standard deviation), mean annual precipitation, and precipitation seasonality at the 30-second spatial resolution. Other environmental characteristics, especially biotic factors such as disease or pests, could also exhibit associations with geography (Woods et al. 2012), but we do not have a good source of data for these.

Study Design Geographic Clines Study.

To test for GCs in germination time and germination success, seeds from 20 source populations distributed across the range of common milkweed (Table S1; Fig. 2) were germinated in controlled conditions in Minnesota (MN) (St. Olaf College, Northfield, MN, U.S.A.) and Virginia (VA) (University of Lynchburg, VA, U.S.A.). We planted seeds from 10 full-sibling genetic families (seed pods) from 5 to 7 different maternal families per source population.

In early March 2020, seeds from 18 source populations were mixed with sand and deionized water and began cold-moist stratification at ~3°C. Due to the COVID-19 pandemic, planting was delayed, so seeds were stratified for more than a year. Consequently, some genetic families stratifying at each location (10 in VA and 17 in MN) experienced either visible mold growth or dry stratification media, and these were excluded from analyses. Seeds from two additional southern source populations were collected in fall 2020 (Table S1) to increase representation of seeds from across the range; we term the initial 18 populations our "core" collection and all 20 populations the "extended" collection. We also collected additional seeds from both MN and VA in 2020 to compare with previous seed collections (2017 and 2018, respectively). Fall 2020 collections were cold stratified for 3–4 weeks prior to planting. In mid-late April 2021, all seeds were planted in M1 potting mix (Grower Select, BFG Supply Co, Burton, OH, U.S.A.) in flats, with each genetic family assigned to 2–4 randomized locations in the growing space. Depending upon availability, 24–48 seeds were planted for each genetic family, totaling ~7,500 seeds planted at each experimental location (MN and VA). Planting took place over a period of 4–8 days; trays were grown in a greenhouse (MN) or indoors (VA) under grow lights (400-watt high pressure sodium bulbs in MN; 250-watt equivalent LED bulbs in VA) set to a 16:8 light:dark cycle and watered with tap water as needed.
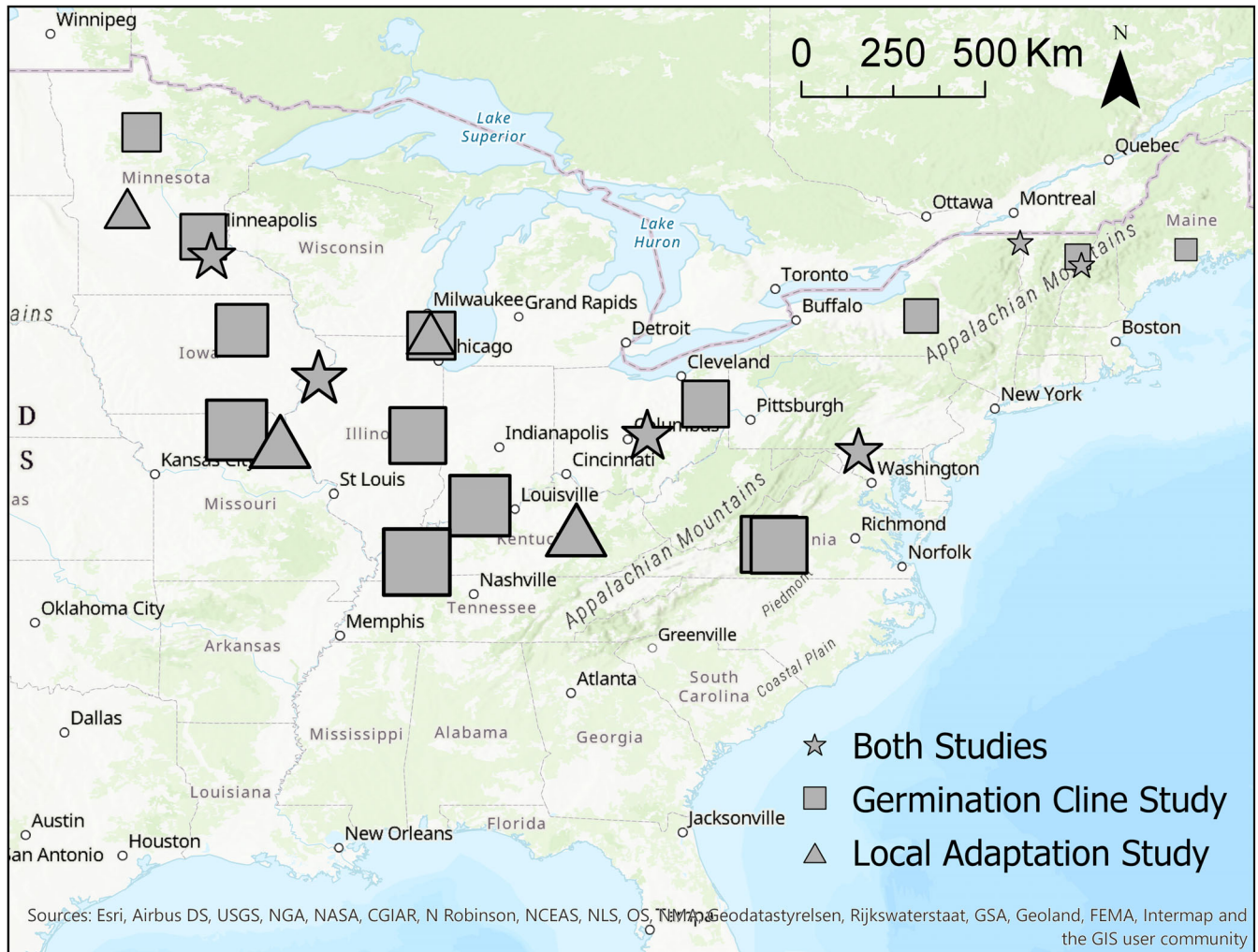
Figure 2. Seed collection sites for the two studies span much of the native range of *Asclepias syriaca* in the Great Plains and Northeast regions of the United States. Squares represent source populations in the germination cline study, triangles represent source populations in the local adaptation study, and stars indicate source populations used in both studies. Symbol size is scaled based on the height predicted for the maternal plants in the population (see "Results" section). Coordinates and dates of collection are reported in Table S1.

In MN, seedling emergence was recorded daily for 18 days after planting, and germination success was calculated as the fraction of planted seeds that emerged. Germination time was measured as the number of days between planting and seedling emergence. In VA, emergence was checked at day 11 and germination success was recorded 21 days after planting. Germination success was higher in VA (see "Results" section), but probably not because of the difference in time until recording: of those seeds that germinated, 95% had done so by day 10 in MN and 97% had done so by day 11 in VA.

Thermal Adaptation Study. To test for adaptation to temperature, in Spring 2019 we planted seeds from 10 populations (Table S1; Fig. 2), as described above and grew them in growth chambers (Conviron CMP 6050, Winnipeg, Manitoba, Canada) set to simulate spring temperatures in Kentucky (KY) and MN,

states from which our most northern and southern seeds were collected. In addition, we manipulated the length of the cold stratification period (CS = 4 or 10 weeks) to test for different dormancy-breaking requirements among the populations, as length of winter varies by location. We pooled seeds from each genetic family within a population for cold stratification, and planted 26 seeds from each source population and cold stratification period into each thermal condition, for a total of 1,040 seeds. We randomly assigned seeds from each site and cold stratification period to locations within a 72-cell plug tray filled with potting mix (SunGro Professional, Agawam, MA, U.S.A.), with four trays in each growth chamber. Given limited space, we planted two seeds of the same type per cell.

We used Online Degree-Day Models (http://uspest.org/cgi-bin/ddmodel.us) to calculate predicted daily max and min temperatures and growing degree days for sites of collection in MN (44.466°N, 93.191°W) and KY (37.839°N, 84.270°W),

based on 10-year historical averages. We selected April 15 as the start date for our simulated spring, and we programmed the growth chamber temperatures to match the average temperatures for the week that centered around that date and each subsequent week for a total of 8 weeks, using a 14:10 hour light:dark cycle (Table S2). Humidity was set to a constant 50% and light was set to maximum at 875 μmols·m^{-2}·second^{-1}. However, checks during the fourth week revealed light intensity below programmed intensity expected in each chamber (~650 in KY and 447 μmols·m^{-2}·second^{-1} in MN), so we adjusted the light intensity of both chambers to 445 μmols·m^{-2}·second^{-1}. Prior research suggests light has little effect on germination rates of common milkweed seeds that have been cold-moist stratified for 2 weeks or more (Baskin & Baskin 1977). Trays were watered approximately every other day as necessary.

Germination, measured as the emergence of the seedling from the soil, and development were observed daily for 8 weeks. If a seed sprouted in a cell that already had a primary seedling, we marked the date of emergence of the younger secondary seedling and uprooted it carefully from the cell. Leaf development time was only measured on primary seedlings and was determined as the difference in days between the time to expansion of the first pair of true leaves and the time of germination.

Statistical Analysis

Geographic and Environmental Predictors. We tested for GCs using latitude and longitude as predictors. Rather than running many additional models with each environmental variable as a predictor, which would inflate the type I error rate, we designed our models to test for GCs. We tested separately for associations between geography and the GIS-extracted climate and soil variables. We used correlation analysis to identify associations that were linear (Pearson) as well as those that were not linear but monotonic (Spearman).

Statistical Models. All analyses were conducted in R version 4.0.2 (R Core team 2020). We used mixed-effects models in the *lme4* package (Bates et al. 2015) to account for nonindependent structure in the data while investigating the fixed effects of geography on traits, including the binomial trait germination success. See Table S3 for the specification of each model. We report significance based on type II sums of squares (Car package; Fox & Weisberg 2019) which is the most powerful approach with unbalanced data when there are not significant interactions. In general, we are not interested in the marginal main effects when interactions are significant (Langsrud 2003).

Maternal Plants. For the 16 accessions with maternal plant data, maternal height and fruit number were modeled as a function of latitude and longitude, accounting for source population as a random variable. Fruit number was square-root-transformed to improve the homoscedasticity of the residuals. For seed-pod-level maternal plant traits, that is, seed number and total seed mass per pod, multiple seed pods could be collected from the same plant, so geographic trends were modeled with the random

variables maternal plant nested within source population. To test for geographic trends in the average mass of individual seeds, we included seed number as a covariate in a model of total seed mass per pod, with maternal plant nested within source population as random variables.

Geographic Clines. We first analyzed data from the core collection, since these were all in cold stratification for the same amount of time. We then followed with the extended collection, for which care with interpretation is warranted.

We tested for GCs in germination success for seeds from each genetic family using a mixed-effects binomial model with mean seed mass, latitude, and longitude as fixed predictors and the following random effects structure: genetic full-sibling family nested within maternal plant within source population within level II ecoregion (Omernik & Griffith 2014; shown in Table S1). Planting site was included as a separate random variable. Latitude, longitude, and seed mass were standardized so estimates could be compared. Because these models were overdispersed, we present quasi-adjusted *p*-values calculated with standard errors scaled by dispersion factor, or the ratio of the sum of squared residuals to the residual degrees of freedom (Dean & Lundy 2016). To characterize the effect of collection year and cold stratification time on germination success, we also used binomial mixed models to compare the early (2017/2018) and late (2020) collections for MN and VA (sites analyzed separately), with genetic family nested within maternal plant as random variables. These models were not overdispersed, so we report significance based on type II sums of squares.

Germination time, measured as days to germination, was square-root-transformed for all analyses. We used mixed models with the same nested structure for random effects; however, because germination time was only measured in the MN greenhouse, we modeled planting tray within the greenhouse rather than planting site as a separate random effect to quantify spatial variation. We used separate mixed-effects models to compare germination time for seeds collected from MN in 2017 versus 2020 and also for seeds collected in VA in 2018 versus 2020; random effects included genetic family nested within maternal plant.

Thermal Adaptation

In the TA study, we used mixed models to test whether germination and growth traits varied in response to latitude, thermal spring conditions (KY vs. MN chambers), cold stratification period (4 vs. 10 weeks), and all two-way interactions. We excluded longitude to avoid overfitting with just 10 source populations. Seeds in this study were pooled by source populations, so source population and planting tray were modeled as separate random variables. For the subset of source populations used in the TA study, seed mass was positively correlated with latitude of origin ($r = 0.84$, $p = 0.0026$), so we did not include seed mass as a covariate. Germination success was analyzed in a binomial mixed model; since each seed was scored independently, we did not test for overdispersion. Germination time

and leaf development time were square-root-transformed for analyses.

Results

Geographic Predictors

Climate and GSL were strongly associated with latitude. High-latitude populations experienced colder temperatures ($r = -0.98$; $p < 0.0001$), less rainfall ($r = -0.79$; $p < 0.0001$), greater seasonality in both precipitation ($r = 0.65$; $p < 0.0001$) and temperature ($r = 0.85$; $p < 0.0001$), and shorter growing seasons ($r = -0.79$; $p < 0.0001$; Table S4; Fig. S1). High-latitude soils tended to be more basic ($r = 0.58$; $p = 0.0046$) and enriched in organic matter ($\rho = 0.50$; $p = 0.017$). Longitude was most strongly associated with seasonality: western sites had greater seasonality in rainfall ($r = -0.74$; $p < 0.0001$) and temperature ($r = -0.51$, $p = 0.01$). Western sites also tended to have lower annual precipitation ($r = 0.41$; $p = 0.04$), higher CEC ($r = -0.43$; $p = 0.045$), and lower organic matter ($r = 0.43$; $p = 0.048$).

Maternal Plants

Maternal plant height showed clines with both latitude (estimate [standard error]: $\beta = -13.48$ [4.47], $\chi^2 = 9.11$, d.f. = 1, $p = 0.0025$) and longitude ($\beta = -11.14$ [4.15], $\chi^2 = 7.19$, d.f. = 1, $p = 0.0043$), such that plants from the south and west were tallest at the time of seed collection (Fig. 2). We did not detect clines in number of fruits per plant (Lat: $\chi^2 = 0.97$, d.f. = 1, $p = 0.33$; Long: $\chi^2 = 0.57$, d.f. = 1, $p = 0.45$) or average seed mass after accounting for the number of seeds in a pod (Lat: $\chi^2 = 0.0008$, d.f. = 1, $p = 0.98$; Long: $\chi^2 = 1.019$, d.f. = 1, $p = 0.31$). However, there was evidence for a longitudinal cline as pods from the east contained lower total masses of seed per pod ($\beta = -0.14$ [0.07], $\chi^2 = 3.96$, d.f. = 1, $p = 0.047$). This

cline might be explained by the number of seeds per pod, which showed a similar but nonsignificant relationship with longitude ($\beta = -15.83$ [9.29], $\chi^2 = 2.90$, d.f. = 1, $p = 0.088$). Overall, these trends suggest that there was geographically structured variation in maternal plant condition.

Geographic Clines Study

Of the ~7,500 seeds planted at each location, 73% germinated in VA and 62% in MN. Seeds from pods with greater average seed mass germinated more successfully (Table S5; Fig. 3). There was no evidence for GCs in the core collection (Table S5; Fig. 3B). The extended collection, however, showed a significant positive relationship between latitude and germination success (Table S5; Fig. 3B).

Across planting sites, only 39% of the seeds from VA that were collected in 2020 germinated, a significantly lower rate than the 87% germination rate of seeds collected in 2018 (contrast = -2.77 [0.55]; $\chi^2 = 26.25$, d.f. = 1, $p < 0.0001$). However, 82% of the MN seeds collected in 2017 germinated, while 68% of seeds collected in 2020 germinated, and this difference was not significant (contrast = -0.68 [0.6]; $\chi^2 = 1.86$, d.f. = 1, $p = 0.17$).

Germination time ranged from 3 to 18 days, with a median of 6 days. Larger seeds were more likely to germinate earlier (Table S5; Fig. 4A), and even after accounting for seed mass, high-latitude seeds germinated earlier in both the core and extended collections (Table S5; Fig. 4B).

Seeds from VA collected in 2018 germinated in an average of 6.66 days, more than 2 days earlier than those collected in 2020 (mean = 8.9 days; contrast = 0.47 [0.14], $\chi^2 = 11.5$, d.f. = 1, $p = 0.0007$). However, seeds from MN collected in 2017 germinated in an average of 6.4 days, and were not significantly different from those collected in 2020 (mean = 6.8 days; contrast = 0.098 [0.08], $\chi^2 = 1.5$, d.f. = 1, $p = 0.22$).
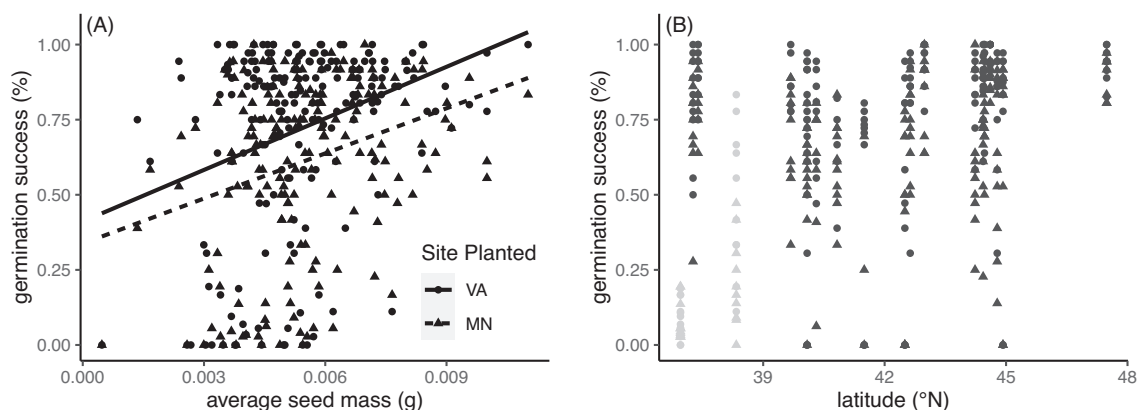

Figure 3. Germination success as a function of seed mass (A) and latitude (B). Data are the proportion of seeds planted from each genetic family that germinated in the grow-room in VA (circles; solid line) or the greenhouse in MN (triangles; dashed line). We studied seeds from 10 genetic families from each of 20 different source populations. (A) Germination success increases with average seed mass (quasibinomial mixed model: $\beta = 0.53$ [0.14], $z = 3.9$, $p < 0.0001$). (B) There is no relationship between geography and germination success in our core collections (see Table S5); however, with the extended collections from two southern sites (in gray) that were cold stratified for a shorter time period, germination success increases with latitude (quasibinomial mixed model; $\beta = 0.69$ [0.3], $z = 2.3$, $p = 0.02$).
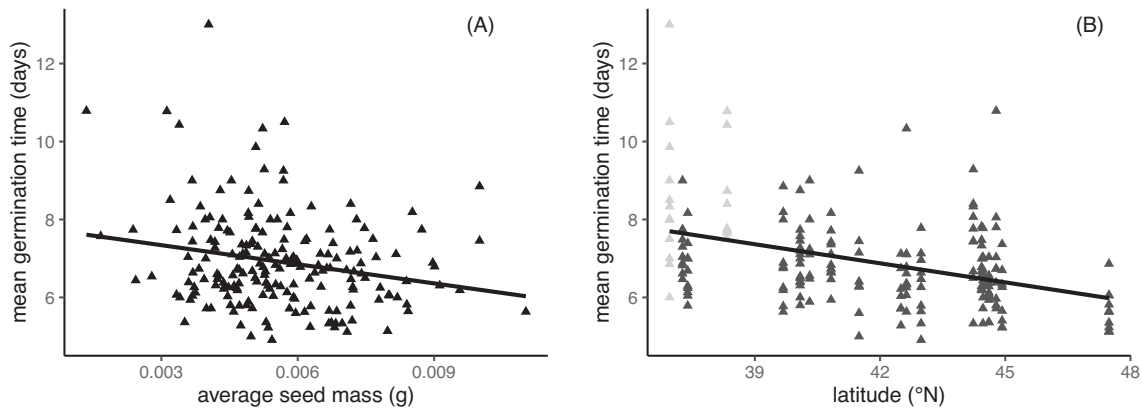
Figure 4. Predictors of germination time in the MN greenhouse. (A) Seed mass. Larger seeds germinate faster ($\beta = -0.035$ [0.11], $\chi^2 = 10.15$, d.f. $= 1$, $p = 0.0014$). (B) Latitude of origin. Seeds originating from high latitude populations germinate faster. The cline is significant whether the two source populations collected in 2020, indicated in gray, that were stored and cold stratified for less time than seeds from other populations are included ($\beta = -0.097$ [0.025], $\chi^2 = 14.88$, d.f. $= 1$, $p = 0.0001$) or excluded ($\beta = -0.062$ [0.021], $\chi^2 = 9.13$, d.f. $= 1$, $p = 0.0025$). Points represent the mean germination time for those individuals that germinated from each genetic family to more clearly reveal patterns. Statistics are from a mixed model of square-root-transformed germination time (see the text).

Thermal Adaptation Study

In the TA study, 589 of the 1,040 seeds that were planted germinated successfully. Germination success among populations in each chamber ranged from 11–92%. Germination success was positively related to latitude (Fig. 5A; $\beta = 0.79$ [0.19], $\chi^2 = 22.47$, d.f. $= 1$, $p < 0.0001$; Table S6). Neither thermal conditions nor cold stratification length affected germination success independently or via an interaction.

In the MN chamber, seeds averaged 32 days to germinate (range of source population means: 29.1–35.6); while in the KY chamber, seeds germinated in 10.6 days on average (range of source population means: 8.7–14.8; thermal spring contrast $= 2.39$ [0.11], $\chi^2 = 567.33$, d.f. $= 1$, $p < 0.0001$; Table S6). We did detect GCs in germination time, but different thermal regimes did not generate crossing clines (Fig. 5B), as would be expected if plants were locally adapted to thermal conditions (Fig. 1A). Under both the MN spring and KY spring conditions, and both cold stratification regimes, seeds originating from higher latitudes germinated more quickly on average (Fig. 5B; $\beta = -0.18$ [0.06], $\chi^2 = 8.05$, d.f. $= 1$, $p = 0.0045$; Table S6), consistent with the results observed in the GCs study. Compared to 4 weeks of cold stratification, 10 weeks of cold stratification tended to result in earlier germination by a little more than 1 day (contrast $= -0.17$ [0.06], $\chi^2 = 13.33$, d. f. $= 1$, $p = 0.0003$; Table S6); there was no interaction between latitude and cold stratification period.

Although germination traits showed clines with latitude, leaf development time did not (Table S6; Fig. 5C). However, it did respond to temperature/chamber (Table S6; Fig. 5C, contrast $= 0.80$ [0.074], $\chi^2 = 143.38$, d.f. $= 1$, $p < 0.0001$). Leaf development took an average of 12 days in the KY chamber (range: 5–24 days), but the colder MN spring conditions extended leaf development time approximately 6 days compared to KY (average $= 17.5$ days; range: 8–33 days).

Discussion

Rapid Germination of Seeds from High-Latitude Populations

As predicted, latitudinal clines in seed and germination traits of *Asclepias syriaca* were common. Seeds from higher latitudes germinate faster: for each degree of latitude, seeds tend to germinate about 0.1 (GCs study) to 0.5 days earlier (TA study). This pattern is consistent with many results showing that high-latitude populations display early phenology (Turesson 1930; Olsson & Ågren 2002; Prendeville et al. 2013) and is thought to be an adaptation to short growing seasons. Woods et al. (2012) found milkweed from high-latitude populations also emerged from rootstock earlier in the season, and early phenology was associated with larger end-of-season biomass in northern but not southern common gardens. Together, these findings suggest that more rapid spring establishment has been selected for in high-latitude milkweed populations.

Contrary to our predictions, we did not find the crossing reaction norms in development time expected in the TA study if plants experience a tradeoff between basal development temperature and thermal time requirements. Instead of germinating faster in thermal spring conditions similar to their source populations, high-latitude seeds always germinated earlier, consistent with adaptation to GSL. Across species, high-latitude populations experience shorter growing seasons and evolve to compensate by developing at colder temperatures, so when grown in a common environment, they develop more quickly (Conover & Schultz 1995; Belk et al. 2005; Toftegaard et al. 2016). High-latitude populations have been shown to germinate faster in other plants, including an herb (*Campanula americana*, Prendeville et al. 2013; Zettlemoyer et al. 2017) and an invasive grass (*Sporobolus alterniflorus*, Liu & Zhang 2020). We hypothesize that for milkweed, rapid germination might be especially important in variable environments
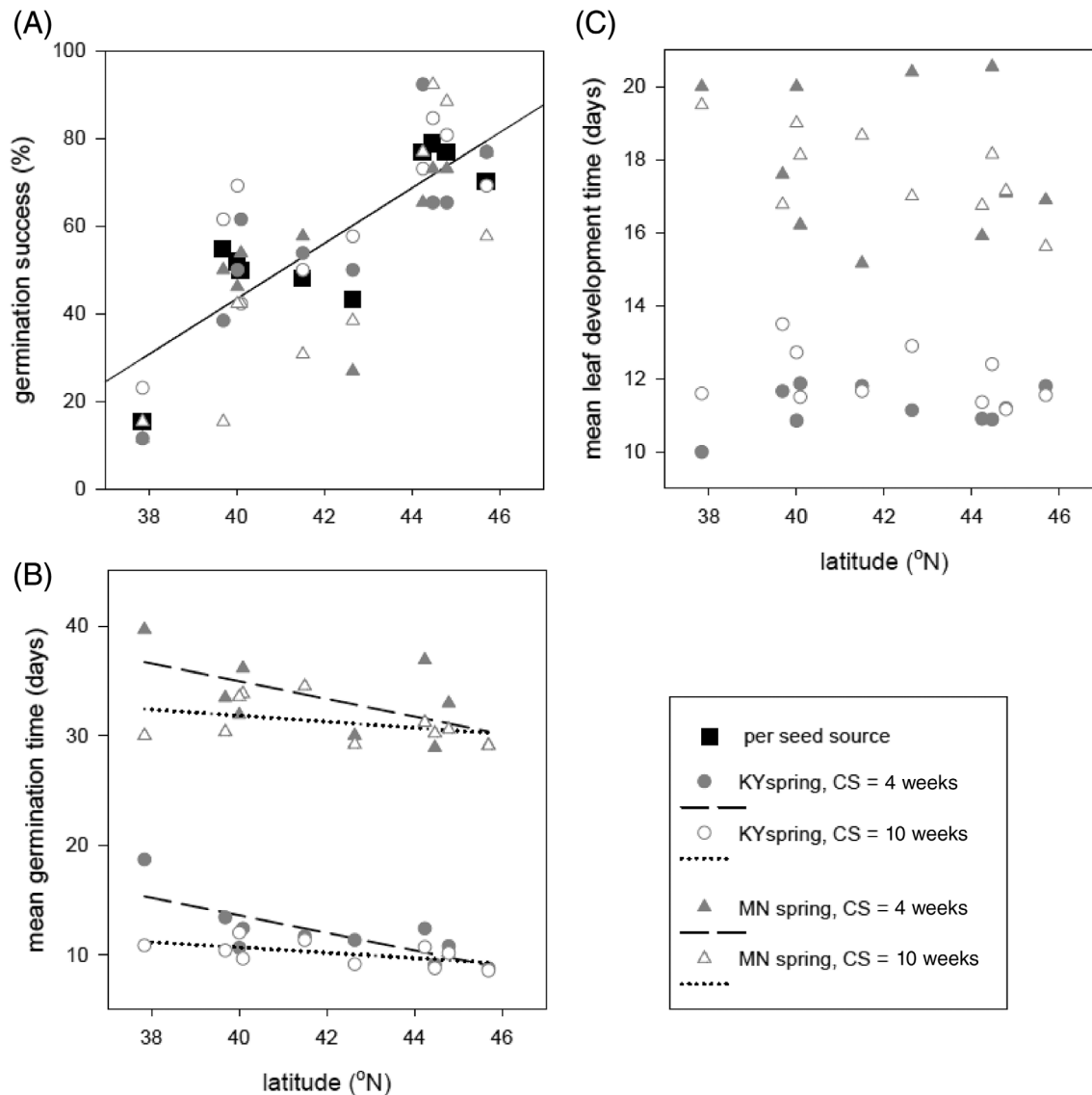
Figure 5. Latitudinal variation in germination success (A), germination time (B), and leaf development time (C) in the local adaptation study. Seeds from 10 populations were exposed to simulated MN or KY thermal spring conditions and cold stratification for either 4 or 10 weeks; plots show population means in each condition. (A) Germination success was highest among seeds from high-latitude populations (binomial mixed model: $\beta =0.79$ [0.19], $\chi^2 = 22.47$, d.f. = 1, $p < 0.0001$) but did not vary with experimentally manipulated environments. (B) Germination was fastest for seeds from high latitudes overall (mixed model: $\beta = -0.18$ [0.06], $\chi^2 = 8.05$, d.f. = 1, $p = 0.0045$; dashed and dotted lines are visual aids only), regardless of whether seeds were grown in KY (circles) or MN (triangles) spring temperatures (interaction: $\chi^2 = 0.2$, d.f. = 1, $p = 0.66$). KY temperatures advanced germination by 22 days compared to MN (contrast = 2.39 [0.11], $\chi^2 = 567.33$, d.f. = 1, $p < 0.0001$). Longer cold stratification reduced germination time by about a day (contrast = -0.17 [0.06], $\chi^2 = 13.33$, d.f. = 1, $p = 0.0003$), but the interaction between cold stratification and source latitude was not significant ($\chi^2 = 0.031$, d.f. = 1, $p = 0.86$). (C) Leaf development time showed no evidence of a cline (mixed model: $\chi^2 = 1.0$, d.f. = 1, $p = 0.32$).

with a limited growing season, perhaps because biomass accumulation facilitates survival through stressful periods (see Stratton 1992), and the risks of germinating early are balanced by the costs of germinating late. Characterizing fitness in the field will be necessary to fully test this idea (Donohue et al. 2010). In a recent study of common milkweed seed establishment in low- and mid-latitude field sites (Missouri and Illinois, U.S.A.), seeds sourced from high-latitude populations germinated earlier, but there was no evidence of an effect of latitude of origin on survivorship or above-ground biomass accumulation by July (Finch et al. 2023). We note that common milkweed invests heavily in below-ground growth and suggest testing whether population differences in germination time correspond with differences in root:shoot ratio, overwintering success or flowering time, especially at high-latitude field sites.

Rapid germination could be achieved through reduced basal temperature or thermal time requirements for germination, reduced dormancy, or reduced demands for dormancy breaking

(Batlla & Benech-Arnold 2015; Fernández-Pascual et al. 2019). We observed a cline in germination time after cold stratification for more than a year, which appeared sufficient to reduce the cline in dormancy, even after accounting for seed mass. Consequently, we infer that high-latitude populations either have lower thermal time or basal temperature requirements for germination only, since clines were not evident for leaf development time. A study of *Festuca altaica* (Canadian fescue) germination showed high-latitude populations had higher basal temperatures and lower thermal time requirements (Qiu et al. 2010). A similar study for milkweeds could clarify whether thermal time underlies the cline in germination time observed.

If seeds do not show adaptation to thermal spring conditions, why do low-latitude populations have consistently later germination times? We expect selection on germination phenology to balance the risks of early germination (prior to the end of winter) and the costs of delayed germination (a competitive disadvantage and less opportunity for growth). Germination timing determines the environment in which seedlings will grow, therefore low-latitude populations may be evolving traits that prevent early germination prior to the end of winter. For example, common milkweed seeds from Illinois and Missouri germinated in simulated fall temperatures (Barton et al. 2020), a phenomenon that is expected to result in the death of a plant over winter. Germination time is also influenced by dormancy: in a study of *Ambrosia artemisiifolia* (common ragweed) populations invading China, high-latitude populations germinated more slowly and also demonstrated greater dormancy (Zhou et al. 2021). Thus, although the direction of clines in germination traits may vary across species (Cochrane et al. 2015), we speculate germination time and dormancy may work together to impact germination phenology.

Inconsistent Clines in Seed Mass and Dormancy

We observed latitudinal clines in seed mass and germination success in one, but not both experiments. When present, these clines were consistent with previous reports of higher germination success (Finch et al. 2018) and higher seed masses (Woods et al. 2012) in high-latitude populations. Limited germination success under otherwise favorable conditions is frequently interpreted as evidence of dormancy (Batlla & Benech-Arnold 2015), but the cline in germination success in the TA study could also be driven by seed mass. Increased seed mass correlates to increased germination success and early seedling survivorship, both within, and across, plant species (Leishman et al. 2000; Moles et al. 2007; Baskin & Baskin 2014). Our GCs study shows that *A. syriaca* is no exception: seeds from genetic families with greater average seed mass germinated earlier and in higher proportions. Similarly, a separate study with individually massed *A. syriaca* seeds (Mohl et al. unpublished data, 2019) provided clear evidence for a positive association between seed mass and germination success ($\beta = 0.48, \chi^2 = 17.73$, d.f. $= 1, p < 0.0001$). Although one study claimed the relationship between population seed mass and germination in *Asclepias syriaca* was weak (Farmer et al. 1986), another demonstrated that germination success, seedling

survival, and seedling performance are all positively associated with individual seed mass, although emergence time was not (Morse & Schmitt 1985). Together, these findings suggest that seed mass could explain the latitudinal cline in germination success observed in the TA study; we found no evidence for a latitudinal cline in germination success in the GCs study in which seed mass and latitude were not confounded, at least when the 2020 seed collections were excluded.

Dormancy could also explain the different patterns of germination success between our GCs and TA studies. The core seed collection of the GCs study, which experienced an extended period of cold stratification, showed no latitudinal cline in germination success. The clear presence of this cline in the TA study, where cold stratification was limited to 10 weeks or less, could be interpreted as evidence that low-latitude populations express greater dormancy that can be overcome by very long periods of cold stratification. Supporting this interpretation, seeds from a low-latitude VA site exposed to 2 years of storage and a full year of cold stratification expressed significantly greater germination success and accelerated germination time compared to seeds from the same site exposed to the more standard 4 weeks of cold stratification. However, 3 years of storage and a full year of cold stratification did not significantly affect either germination trait for seeds from high-latitude MN. Other studies have also documented higher dormancy in low-latitude milkweed populations, as demonstrated by longer periods of cold stratification being necessary to germinate a given proportion of seeds and by a lower proportion of seeds germinating (Finch et al. 2018). It would be interesting to investigate whether high levels of dormancy in low-latitude populations allow viable seeds to persist in a seed bank in the soil for more than a year.

Many studies report higher dormancy in high-altitude or high-latitude populations as a means to avoid germinating in unfavorable winter conditions (Meyer et al. 1995; Cavieres & Arroyo 2000; Fernández-Pascual et al. 2013), so reduced dormancy in high-latitude populations is relatively unexpected. Dormancy preserves seeds until environmental cues signal a favorable environment for germination (Finch-Savage & Leubner-Metzger 2006), but it may also serve to spread risk in temporally variable environments (Venable & Brown 1988). However, we found no evidence that latitude was correlated with the coefficient of variation in germination time (GCs study: $r = 0.069, T = 0.29$, d.f. $= 18, p = 0.77$; TA study: $r = -0.16$, $T - 0.47$, df $= 8, p = 0.65$), suggesting that latitudinal variation in dormancy is more likely to facilitate environmental tracking than risk spreading. Diurnal fluctuations are likely to facilitate germination by terminating dormancy (Farmer et al. 1986; Batlla & Benech-Arnold 2015). We suspect that dormancy might be less important in high-latitude sites where snowpack dampens diurnal fluctuations in light and temperature until spring. In contrast, low-latitude populations might benefit from deeper dormancy that reduces the risks of germinating during the fall (Bandara et al. 2019) or under false spring conditions (Ojala 1985; Meyer et al. 1995; Fowler & Dwight 1964; Schütz & Milberg 1997). In fact, as common milkweed expands its range farther south (Wyatt et al. 1993; Wyatt 1996), southern populations may be evolving increased dormancy to facilitate

germination at appropriate times. Although we found no evidence for local adaptation to spring thermal conditions, milkweeds might display adaptations to local overwintering conditions; specifically, rapidly germinating seeds from high-latitude populations might germinate and perish before the end of winter at higher frequencies in low-latitude sites, while dormant seeds from low latitudes might be outperformed by early-germinating seeds at northern sites. If true, changing climates that reduce the length of winter and change the pattern of exposure to diurnal temperature fluctuations could impact milkweeds (Walck et al. 2011; Bandara et al. 2019), though more data are necessary to make precise predictions.

Unlike Finch et al. (2018), we found no effect of thermal condition or cold stratification period on germination success in our TA experiment. We surmise that gradual warming over 8 weeks in our study, rather than incubation for 2 weeks as in Finch et al. (2018), meant that differences in relative dormancy (the range of environmental conditions sufficient to allow germination; Betlla and Benech Arnold 2015), were expressed as differences in germination time rather than germination success. That is, seeds with greater relative dormancy experienced a suitable environment for germination later in time, but were still able to germinate. Indeed, increasing the cold stratification period, which was intended to break dormancy, reduced the time necessary for germination. However, 10 weeks of cold stratification was insufficient to remove the latitudinal cline in germination success. Since seed mass is predicted to be inversely related to dormancy (Venable and Brown 1988, Leishman et al. 2000), both dormancy and seed mass could jointly contribute to the pattern of larger seeds from higher latitudes germinating at a higher rate in the TA study.

Interpreting Variation in Germination Traits

Although we observed population differences, especially in germination time, phenotypic plasticity in milkweed may be more likely to impact restoration success. For example, warmer spring temperatures strongly advanced milkweed germination time in our TA study. However, warmer winters might delay the time when monarch butterflies migrate north from Mexico because they respond to prolonged exposure to cold and possibly photoperiod cues (Guerra and Reppert 2015). Indeed, citizen science data document a shift across 18 years of observations toward earlier milkweed emergence, but later monarch arrival (Howard and Davis 2015). As a result, as the climate warms, milkweeds might be older, better-defended, and/or already colonized by competitors or predators when monarchs first arrive in the spring (Yang and Cenzer 2020). Mowing milkweed could reset growth with possible benefits for monarchs (Haan and Landis 2020).

Because seeds were collected from wild populations rather than plants grown in a common garden, we cannot confirm that clines we observed are adaptive rather than plastic responses to the maternal environment, which strongly influences seed traits (Fernandez-Pascual et al. 2013; Baskin and Baskin 2014). Interestingly, many of the maternal plant traits demonstrated longitudinal clines that were not present in our GCs study. Of the

maternal traits studied, only height showed a latitudinal cline, though seed mass also showed a cline in a subset of the populations. Based on these findings, it is possible that southern plants grow taller in response to competition but also experience greater stress, especially at the range edge, thereby producing smaller seeds that germinate less well. Even if the clines we detected are genetically based, they might not be detectable in all environments (Alba et al. 2016). As such, germination studies under field conditions, where water stress, herbivory, competition, and overwintering success can impact germination success, are an important next step to generate relevant predictions for plant establishment.

Currently, there is scant evidence from field studies supporting local seed sourcing. However, growing conditions in a given year may muddle evidence of local adaptation that can be resolved with longer-term data (Barton et al. 2020). Given repeated findings of latitudinally based clines in common milkweed seed and germination traits, climate, and especially GSL, latitude is likely an important driver of population differentiation in common milkweed. We provisionally recommend sourcing milkweeds seeds from similar climates. However, we hesitate to recommend climate-matching seeds from sources similar to predicted future climates (Weeks et al. 2011, Breed et al. 2013). Low-latitude seeds often germinated at lower frequencies; therefore, it does not yet appear they will be more successful than locally-sourced northern seeds. As the climate warms, the discrepancy between high- and low-latitude germination success may increase if shorter winters fail to break higher dormancy demands in low-latitude seeds (Walck et al. 2011); alternatively, low-latitude populations may harbor the dormancy necessary to prevent premature germination in a warmer world. At the same time, range-edge populations may experience reduced fitness. Future research will be necessary to determine the value of low-latitude seed sources for common milkweed restoration. Although high-latitude seeds could initially be successful in more southern springs, since faster germination may translate into long-term benefits for growth and reproduction (Verdú and Traveset 2005), data about fitness at all life stages, including overwintering success, are necessary to predict long-term outcomes and to optimize milkweed restoration practices in the face of climate change.

Acknowledgments

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Supporting Information

The following information may be found in the online version of this article:

Table S1. Seed collection sites for each study.
Table S2. Growth chamber settings for the thermal adaptation study.
Table S3. Model specifications
Table S4. Environmental correlations.
Table S5. Statistics for the geographic cline study.
Table S6. Statistics for the thermal adaptation study.
Figure S1. Environmental correlation plots.