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A transcontinental experiment elucidates (mal)adaptation of a cosmopolitan plant to climate in space and time

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Data are provided for peer review. All data involved in this study is available on the GitHub page for LJA (https://github.com/ljalbano/transcontinental_common_garden).

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Abstract (350 words)

Climate change and the global spread of invasive species are two of the most significant threats to biodiversity. Both of these phenomena subject populations to novel conditions, either in space (species invasion) or in time (climate change), yet the role of adaptation in how populations respond to these rapid environmental shifts is poorly understood. We conducted a large-scale trans-continental common garden experiment using white clover (*Trifolium repens*, Fabaceae) to test if adaptive evolution to spatiotemporal variation in climate could contribute to the ecological success of one of the most widespread invasive plant species in the world. Individuals from 96 populations of *Trifolium repens* (white clover) from both its native (Europe) and introduced (North America) ranges were planted into four experimental common gardens located in northern (Uppsala, Sweden) and southern (Montpellier, France) Europe, and northern (Mississauga, Canada) and southern (Louisiana, USA) North America. We recorded plant sexual and clonal fitness in each common garden and assessed whether the strength of local adaptation differed between the native and introduced ranges and whether populations are rapidly adapting to climate change. Results show that local adaptation was only evident when populations were transplanted into common gardens located in the same range (native or introduced) from which they originated and was driven by stronger selection (due to climatic factors rather than herbivory) at lower latitudes in both ranges. Our results indicate that rapid local adaptation across a large latitudinal gradient has occurred in *T. repens* populations in less than 400 years since its introduction to North America. However, we find evidence of an adaptation lag in the northern common garden in the introduced range, with plants from historically slightly warmer climates exhibiting the greatest fitness. These findings support two major conclusions: 1) white clover can rapidly adapt to spatial variation in climate in its introduced range as well as the

native range, and 2) despite rapid adaptation to novel ranges, introduced white clover populations are not keeping pace with rapid climate change. Overall, our results provide insight into the role of adaptation in facilitating the ecological success of invasive species in a rapidly changing world.

Introduction

Natural selection results in populations evolving traits that are advantageous to the environmental conditions of a particular location, a phenomenon known as local adaptation (Turesson, 1922; Kawecki and Ebert, 2004; Hereford, 2009). A fundamental aspect of local adaptation is that variation in biotic and abiotic factors leads to changes in the relative fitness of genotypes between environments (Gillespie and Turelli, 1989; Kawecki and Ebert, 2004; Lee and Mitchell-Olds, 2011). Such genotype-by-environment interactions can result in fitness trade-offs, whereby genotypes exhibit higher relative fitness in their local environment and lower fitness in other environments (Gillespie and Turelli, 1989; Hereford, 2009; Leimu and Fischer, 2008; Savolainen et al., 2013). However, while environmental variation can result in local adaptation, it remains poorly understood how locally adapted populations are affected when environments rapidly change in space or time (Kawecki and Ebert, 2004; Anderson and Song, 2020). Human activities are exposing populations to novel environments, both across space through species introductions (Pyšek et al., 2020), and over time through anthropogenically mediated climate change (Hoffmann and Sgrò, 2011). Understanding how populations simultaneously adapt to novel biotic and abiotic environments over space and time remains an important problem in evolutionary ecology (Hendry, 2017; Siepielski et al., 2017), and this understanding is necessary to determine how increasingly pervasive anthropogenic disturbances

are altering the ecology and evolution of native and introduced species (Parmesan, 2006; Fugère and Hendry, 2018).

For a species to successfully become invasive in a novel range, an introduced population must overcome substantial barriers to its establishment and proliferation. Introduced populations are frequently subject to shifts in selection pressure compared to the native environment to which they are locally adapted (Erfmeier, 2013; Colautti and Lau, 2015; Lau and terHorst, 2015). This shift in selection pressure could involve greater stress imposed by the biotic environment (e.g., novel species interactions), or the abiotic environment (e.g., climatic factors), which could inhibit the spread of an introduced population (Erfmeier, 2013; Colautti and Lau, 2015; Lau and terHorst, 2015). Additionally, classic hypotheses in invasion biology propose that inherent characteristics of introduced populations will limit their ability to respond to these novel selection pressures (Allendorf and Lundquist, 2003; Estoup et al., 2016). For example, small, introduced population sizes can result in Allee effects, such as mate limitation leading to limited reproductive success for obligate outcrossers (Taylor and Hastings, 2005; Burns et al., 2011; Pannell et al., 2015). Furthermore, small population size post-introduction can impose genetic bottlenecks (Tsutsui et al., 2000). Such evolutionary events have the potential to reduce fitness through inbreeding depression or limit genetic diversity within an introduced population to the point that stressful, novel selection pressures cannot be overcome (Tsutsui et al., 2000; Kolar and Lodge, 2001; Lau and terHorst, 2015). In most cases, the ecological and/or genetic constraints experienced by introduced populations are sufficient to result in the failure of those populations to establish and their eventual extirpation (Kolar and Lodge, 2001; Jeschke and Pyšek, 2018).

Despite the challenges presented by introduction to novel environments across space, introduced populations are also presented with substantial ecological opportunity if genetic

constraints can be overcome. For example, selection regimes experienced in novel biotic and abiotic environments could differ in a manner that is either benign or even advantageous to an introduced population compared to its native range (Erfmeier, 2013; Colautti and Lau, 2015). Classic hypotheses in invasion biology, while not universally supported, posit that a release of an introduced species from its natural enemies in the native range (Keane and Crawley, 2002), the evolution of increased competitive ability relative to native species (Blossey and Nötzold, 1995), or the possession of biochemical compounds that are novel to potential enemies in an introduced range (Callaway and Ridenour, 2004), could each result in the proliferation and success of an invader. It is also possible that introduced populations do not exhibit strong evidence of bottlenecks due to reduced genetic diversity (Colautti and Barrett, 2013; Battlay et al., 2024). This could occur because repeated introductions of novel genotypes can increase effective population size, or because reductions in genetic variation were not substantial enough to limit the ability of a population to respond to novel selection pressures to begin with (Maron et al., 2004; Dlugosch and Parker, 2008a; Dlugosch and Parker, 2008b; van Boheemen et al., 2017). In fact, when introduced populations experience strong selection in novel environments, it could facilitate rapid adaptation to those novel environments, which could in turn, contribute to the invasion success of a species (Maron et al., 2004; Dlugosch and Parker, 2008a; Colautti and Barrett, 2013; Colautti and Lau, 2015; Simón-Porcar et al., 2021; but see Genton et al., 2005). However, the extent to which rapid local adaptation to novel biotic and abiotic conditions can facilitate the success of a widespread invasive species across large environmental gradients remains poorly understood.

While there are examples in which introduced populations have rapidly adapted to novel environments across space, it is important to consider that these novel environments may also be

changing through time. Temporal shifts in a local environment can cause selection pressures to fluctuate, which could result in traits that were previously adapted to that environment becoming maladaptive (Wilczek et al., 2014; Aguirre-Liguori et al., 2019; Exposito-Alonso et al., 2019; Anderson and Wadgymar, 2020; Bontrager et al., 2020). If the rate of adaptation is exceeded by the rate of environmental change, this disruption of local adaptation is likely to result in reduced fitness (i.e., maladaptation) of populations (St Clair and Howe, 2007; Atkins and Travis, 2010; Wilczek et al., 2014; Aguirre-Liguori et al., 2019). Recent global warming may result in populations becoming maladapted to their local climate conditions, whereas populations from lower latitudes or altitudes become better suited to environmental conditions at higher latitude or altitudes; a phenomenon known as an adaptation lag (Wilczek et al., 2014; Kooyers et al., 2019; Anderson and Wadgymar, 2020). Currently, the frequency and magnitude of adaptation lags are poorly understood and require large scale experiments with sufficient population sampling along broad environmental gradients to assess local (mal)adaptation as a result of rapid climate change (Anderson and Song, 2020).

If invasive species rapidly adapt to novel environments across space, similar rapid adaptation to climate change through time could facilitate their continued range expansion, persistence, and pervasiveness (Byers, 2002; Maron et al., 2004; Colautti and Barrett, 2013; Oduor et al., 2016). In fact, invasive species are often better at climate tracking and more resistant to climate warming than native species, which could contribute to their success as invaders (Wolkovich et al., 2013). However, the interplay between local adaptation, species introductions, and anthropogenically mediated climate change remains an understudied, yet critically important, aspect of understanding the ongoing human-induced biodiversity crisis (IPBES, 2019). This problem is best addressed through field experiments testing local adaptation

in both the native and introduced ranges of widespread invasive species, alongside elucidating the environmental conditions that may result in rapid adaptation and invasion success (Colautti and Lau, 2015; Anderson and Song, 2020).

Plant-herbivore interactions have been highlighted as key selective factors that determine patterns of local adaptation, species responses to environmental change, and biological invasions. Local herbivore communities impose selection through preferential feeding on plants with lower resistance, influencing plant defense evolution through an evolutionary arms race (Ehrlich and Raven, 1964; Agrawal, 2007; Züst et al., 2012). Spatial variation in herbivore communities is therefore likely to lead to spatial variation in plant defense traits (Anstett et al., 2018; Baskett and Schemske, 2018; Moreira et al., 2018). For example, the latitudinal herbivory-defense hypothesis predicts greater diversity and abundance of plant defenses at lower latitudes due to the presence of greater herbivore diversity and abundance (Coley and Barone, 1996; Johnson and Rasmann, 2011; Anstett et al., 2016; Moreira et al., 2018). Plant defense evolution is also likely to be driven by the abiotic environment through growth-defense trade-offs (Coley et al., 1985; Herms and Mattson, 1992; Züst and Agrawal, 2017). The metabolic costs of defenses can be substantial, while abiotic environments and the stressors they impose can influence the availability of resources to plants, exacerbating defense costs (Coley et al., 1985; Fine et al., 2006; Hahn et al., 2019). Furthermore, it is increasingly recognized that anthropogenic activity can influence the evolution of plant defenses (Bidart-Bouzat and Imeh-Nathaniel, 2008; Massad and Dyer, 2010; Miles et al., 2019). Climate warming could result in increased herbivore feeding and/or increased plant defenses (Dostálek et al., 2020; Lemoine et al., 2013) or could contribute to invasion success by decoupling locally adapted plant-herbivore interactions (Adams et al., 2009; Costan et al., 2022; but see Agrawal and Kotanen, 2003). It is therefore important to understand how

populations are adapting to their local biotic and abiotic environments and how anthropogenic disturbance can affect adaptation.

An ideal study system to test questions surrounding adaptation to novel environments post-introduction and in response to climate change would be a widespread and well-documented invasive species that is economically and ecologically important. It would also be advantageous to have a system with well known agents of biotic and abiotic selection across space and over time that can drive adaptation in the native and introduced ranges. The cosmopolitan plant white clover (*Trifolium repens* L.) offers such a system. *Trifolium repens* is an allopolyploid perennial legume that is native to Eurasia and has been introduced to inhabited continents throughout the world, including North America (Daday, 1958; Kjærgaard, 2003; Griffiths et al., 2019). Mechanisms of biotic and abiotic selection in *T. repens* have been studied for over 70 years, often in the context of the formation of clines – a common manifestation of local adaptation represented by gradual changes in phenotypes or genotypes across environmental gradients (Endler, 1977; Stinchcombe et al., 2004; Savolainen et al., 2013).

In *T. repens*, clines in a prominent chemical defense mechanism (cyanogenesis) have been identified across many types of environmental gradients (Daday, 1954a; Daday, 1954b, Daday, 1958; Till, 1987; Kooyers and Olsen, 2013; Kooyers et al., 2014; Thompson et al., 2016), providing opportunities to test hypotheses surrounding the involvement of cyanogenesis in adaptation to environmental change. Specifically, latitudinal clines in the native European range of *T. repens* exhibit fixation of cyanogenesis in warmer climates and fixation of acyanogenesis in cooler climates (Daday, 1954a). Similar, but shallower, clines have also been observed in many introduced ranges of *T. repens*, possibly indicating weaker local adaptation post-introduction, which occurred less than 400 years ago in the case of North America (Daday, 1958; Kjærgaard,

2003; Kooyers and Olsen, 2012; Innes et al., 2022). Alternatively, recent studies identify a lack of involvement of cyanogenesis in local adaptation, and instead provide evidence for other candidate traits that are likely to vary across environmental gradients in driving local adaptation, such as flowering time and investment in vegetative growth versus reproduction (Wright et al., 2018; Wright et al., 2021; Kuo et al., 2024). However, no study has directly compared the strength of local adaptation in the native and introduced ranges of *T. repens* or directly tested whether *T. repens* populations exhibit a spatial lag in adaptation to climate change.

To understand adaptation to environmental variation in an invasive species over space and time, we conducted a trans-continental common garden field experiment across latitudinal gradients in both the native (Europe) and introduced (North America) ranges of *T. repens*. We first address four research questions surrounding whether *T. repens* can adapt to environmental variation in space. (Q1) Are *T. repens* populations locally adapted to their climate of origin and does the strength of local adaptation differ across space? This question is a prerequisite condition before any comparisons can be made regarding local adaptation between the native and introduced ranges. (Q2) Does *T. repens* exhibit stronger local adaptation in its native range than in its introduced range? We anticipate weaker local adaptation in the introduced range based on the shorter amount of time that introduced populations have had to locally adapt to novel environments in North America (less than 400 years). (Q3) What are the roles of cyanogenesis and herbivory (i.e., biotic factors) in driving local adaptation across space? (Q4) What bioclimatic variables (i.e., abiotic factors) have the strongest impact on fitness across space (latitudes and ranges) in *T. repens*? Answering the latter two questions would help to identify the biotic and abiotic environmental drivers of local adaptation. We then focus on whether *T. repens* can adapt to climate change through time: (Q5) Are there adaptation lags to climate change in *T.*

repens, such that populations originating from warmer and/or drier climates exhibit greater fitness than local populations? This study is among the first to investigate the ability of an introduced species to rapidly adapt to novel environments in space and time simultaneously. Our results elucidate the potential for rapid adaptation to contribute to initial invasion success and to continued ecological success of invaders in the face of ongoing environmental change, while furthering our understanding of local adaptation to climate and the biotic environment.

Methods

Study system

Trifolium repens has been a model for the study of plant defense based on its polymorphism for the production of the potent chemical defense hydrogen cyanide (HCN; Hughes, 1991). In fact, the ability to produce HCN as an antiherbivore defense may have contributed to the range expansion of *T. repens*, allowing it to thrive in human-disturbed habitats such as lawns, fields, and roadsides in many regions throughout the world (Burdon, 1983; Kjærgaard, 2003; Olsen et al., 2021). Cyanogenesis occurs through the hydrolyzation of cyanogenic glucosides by the enzyme linamarase. In *T. repens*, the presence or absence of cyanogenic glucosides, linamarin and lotaustralin, is controlled by three linked genes in their metabolic pathway (*CYP79D15*, *CYP736A187*, and *UGT85K17*; referred to collectively as *Ac*/*ac*), while the presence or absence of the enzyme linamarase is controlled by the gene *Li*/*li* (Olsen et al., 2007; Olsen and Small, 2018). Thus, four phenotypes exist for HCN and the two metabolic components required for its production: one in which HCN is constitutively produced (AcLi) and three that are acyanogenic and therefore lack the defense entirely (acLi, Acli, acli; Corkill, 1940; Atwood and Sullivan, 1943).

Cyanogenesis is widely considered an adaptive polymorphism because *T. repens* exhibits clines in the presence of HCN across numerous types of environmental gradients (Daday, 1954a; Daday, 1954b; Daday 1958; Brighton and Horne, 1977; Thompson et al., 2016; Innes et al., 2022; Santangelo et al., 2022). In general, dominant *Ac* and *Li* alleles (and thus the ability to produce HCN) are fixed in warmer climates, while recessive *ac* and *li* alleles are fixed in colder climates (Daday, 1954a; Daday, 1954b; Daday, 1965). One hypothesis to explain cyanogenesis clines is a gradient in herbivory, with greater diversity and abundance of herbivores in warmer climates resulting in stronger selection favouring cyanogenic plants (Angseesing, 1974; Dirzo and Harper, 1982; Kakes, 1989). Alternatively, empirical studies across various environmental gradients have identified temperature, soil moisture, and soil nutrients as abiotic factors that may be involved in cyanogenesis cline formation (Daday, 1954a; Daday, 1965; Gleadow and Woodrow, 2002; Kooyers et al., 2014; Kooyers et al., 2018). Overall, despite 70 years of research on the drivers of cyanogenesis clines in *T. repens*, there remains little consensus on how variation in environmental conditions influence selection on cyanogenesis in *T. repens* or if cyanogenesis is primarily responsible for rapid local adaptation to novel environments (Daday, 1954a; Daday, 1965; Hughes, 1991; Kooyers and Olsen, 2013; Kooyers et al., 2014; Wright et al., 2018; Wright et al., 2021; Albano and Johnson, 2023; Kuo et al., 2024). Our study sought to help resolve this debate by directly assessing cyanogenesis and bioclimatic factors as potential drivers of local adaptation in *T. repens*.

Sampled populations and common garden sites

We addressed our research questions using four common gardens distributed across northern (Uppsala, Sweden) and southern (Montpellier, France) regions of the native European range of *T. repens*, and northern (Mississauga, ON, Canada) and southern (Lafayette, LA, USA) regions of

the North American introduced range (**Figure 1**). Seeds were collected from 49 natural *T. repens* populations scattered across the native European range spanning ~27° of latitude, and 47 natural *T. repens* populations along a 2500 km north-south transect spanning ~21° of latitude in the introduced North American range (**Figure 1**). To minimize maternal and epigenetic effects, we hand-pollinated plants germinated from field-collected seeds in a growth chamber environment (25°C day, 15°C night, 14 h:10 h light:dark cycle; Conviron MTPS, Winnipeg, MB, Canada) to create an outbred F1 cohort of seeds for each population. F1 generation seeds were germinated and established for four weeks in peat pots (8 cm diameter, 591 mL volume) in uniform growth chamber or greenhouse conditions near each common garden site. Five replicate individuals from each population were transplanted in peat pots directly into the natural soil of a typical cultivated lawn at each common garden site. Inconsistent germination success resulted in some populations not being included in some common garden locations, and in very limited cases, populations had four or six individuals planted. The resulting total number of plants per garden was 465, 465, 458, and 470 in Mississauga, Lafayette, Uppsala, and Montpellier, respectively. Plants were arranged in rows and columns with 1 m spacing, and black landscaping fabric was used to cover the entirety of the lawn to prevent the rooting of stolons into the natural soil between plants. 12 cm diameter holes were cut in the fabric for each individual plant, leaving a 2 cm ring of natural vegetation around each plant to allow for natural competition. This vegetation was trimmed monthly throughout the experiment. Planting dates matched growing seasons in each location. The Lafayette and Mississauga common gardens were established in April and May 2020, respectively, while the Montpellier and Uppsala common gardens were postponed due to COVID-19 restrictions in Europe, leading to their establishment in March and May 2021, respectively. Plants were given supplemental water for the first two weeks of the experiment and

intermittently after the first two weeks when necessary to avoid excessive mortality. Plants that died within the first two weeks of the experiment were deemed to have died due to transplantation stress and were replaced using extra germinated individuals from the same population. Plants that died after two weeks were deemed to have died due to the local environmental conditions at each common garden site and were not replaced. Each common garden was maintained for two full growing seasons.

Tissue collection, herbivory estimates, and measures of fitness

After 1-2 months of growth, one to three fully-expanded leaves were harvested from each surviving individual and frozen at -20°C. These leaves were later freeze-dried and used for DNA extraction, followed by low coverage whole genome sequencing to predict the presence of *Ac/ac* and *Li/li* alleles (see **Supporting Information**). In total, 586 individuals (Mississauga: 302, Lafayette: 99, Uppsala: 78, Montpellier: 107) were successfully sequenced and characterized as possessing either dominant or recessive alleles at *Ac/ac* and *Li/li.* The remaining 1272 individuals were not genotyped because they either died before tissue could be harvested or genomic library preparations were unsuccessful. Visual herbivory estimates (in % leaf area consumed) were conducted monthly following the methods of Johnson et al. (2016). Herbivory was analyzed for the month exhibiting peak herbivory within each garden. Multiple components of plant fitness were measured throughout the growing seasons at each location. Survival and onset of flowering were assessed weekly during the first growing season. Mature fruits were counted and collected weekly, and seeds were separated using a thresher (Precision Machine Co., Lincoln, NE, USA) to obtain total seed set mass for each plant across both growing seasons of the experiment. Photos were taken of each plant monthly during the first growing season at each common garden location and assessed for plant area using Easy Leaf Area (Version 2.0; Easlon

and Bloom, 2014). The maximum plant area was then determined and the growth rate across the first growing season was calculated as $(\ln[A_{max}] - \ln[A_1])/(t_{max} - t_1)$, where A_{max} represents the maximum plant area, A_1 represents the initial plant area (i.e., after one month of growth in the common garden), and $(t_{max} - t_1)$ represents the number of days between photos taken for maximum plant area and initial plant area.

Calculating climatic distance

Six bioclimatic variables were extracted from WorldClim (Version 2.1, February 2023) at a resolution of 30 seconds for each original *T. repens* population and common garden site (O'Donnell and Ignizio, 2012). The variables included were mean annual temperature ("BIO1"; MAT), maximum temperature of the warmest month ("BIO5"), minimum temperature of the coldest month ("BIO6"), annual precipitation ("BIO12"), precipitation of the wettest month ("BIO13"), and precipitation of the driest month ("BIO14"). The extracted values of each bioclimatic variable for each common garden location can be found in **Table S1**. Values for all six variables were z-score standardized to allow for analysis on an equivalent scale. A principal component analysis was performed using the *princomp* function in R, with dimensions 1, 2, and 3 explaining 65.8%, 22.1%, and 9.1% of the variation, respectively (**Figure S1**). Coordinates for each location of origin and each common garden location from all three dimensions were then extracted using the *get_pca_ind* function in the *factoextra* package (version 1.0.7; Kassambara and Mundt, 2020) and then plotted on a 3D graph (**Figure S2**). The weighted Euclidean distance between points in 3D space was calculated using the formula:

$$Distance = \sqrt{\sum \left(w_i \left[O_{ij} - G_i \right]^2 \right)} \quad (1)$$

Where w_i represents the proportion of variance explained by the ith dimension, O_{ij} represents the value of the jth population of origin in the ith dimension, and G_i represents the value of a given

common garden in the *i*th dimension. We used this weighted Euclidean distance as a measure of the climatic distance between the location of each population of origin and each common garden location based on our six bioclimatic variables of interest (Samis et al., 2019). This measure of climatic distance was then used as a predictor variable of plant fitness in our statistical analyses.

Statistical analysis

All statistical procedures and analyses were conducted in R Version 4.1.2 (2021) and RStudio Version 2022.12.0 (R Core Development Team, 2022). Seven response variables were assessed as components of fitness: i) whether or not plants survived the first growing season, ii) whether or not plants flowered in the full experiment, iii) whether or not plants produced seeds in the full experiment, iv) number of flower heads produced in the full experiment, v) seed set mass produced in the full experiment, vi) maximum plant area in the first growing season, and vii) growth rate in the first growing season. Herbivory during the first growing season was also measured as an additional non-fitness response variable. The number of flower heads and seed set mass were both zero-inflated, which led to the use of a two-step modelling approach as described previously (Albano and Johnson, 2023). Briefly, both variables were first analyzed in binomial models to assess whether or not plants produced flowers or seeds. Non-zero flower head numbers and seed set masses were then subsequently analyzed as separate measures of fitness. All general linear mixed effects models were conducted using the *glmmTMB* function in the *glmmTMB* package (version 1.1.5; Brooks et al., 2017). All test statistics and *P* values were calculated using the *Anova* function in the *car* package (version 3.0-12; Fox and Weisberg, 2019) with Type II sums-of-squares. For models using quantitative measures of fitness, all transformations necessary to meet assumptions of normality and homogeneity of variance can be found in **Table S2**.

Variation in the strength of local adaptation between common garden locations

To determine whether or not *T. repens* populations exhibit local adaptation and to assess the strength of local adaptation at each garden location (Q1), all response variables were analyzed using the following structure:

$$Response \sim Continent\ of\ Origin\ (O) + Garden\ Location\ (L) + Climatic\ Distance\ (C) + O{:}L + O{:}D + L{:}D + O{:}L{:}D + (1|Population) \quad (2)$$

Continent of origin (North America or Europe), garden location (Mississauga, Lafayette, Uppsala, or Montpellier), and climatic distance (the combined measure of six bioclimatic variables of interest described above) were included as fixed effects, along with all two- and three-way interactions. Population nested within continent of origin was included as a random effect to account for non-independence of replicates within each population. An interaction between continent of origin and climatic distance would indicate that the strength of local adaptation varies based on whether or not populations originated in the native or introduced range. An interaction between garden location and climatic distance would indicate that the strength of local adaptation varies based on the particular common environment in which it is being tested. The strength of local adaptation refers to the magnitude of the fitness advantage for local populations compared to populations transplanted an increasingly larger climatic distance from their origin. We therefore interpret the slope of the relationship between climatic distance and fitness as being representative of the strength of local adaptation within a given common garden.

Variation in the strength of local adaptation between ranges

To assess the strength of local adaptation between the native and introduced ranges of *T. repens* (Q2), the analyses of the previous sub-section were repeated, with the continent of the common garden (North America or Europe) replacing the location of the common garden in the models:

$$Response \sim Continent\ of\ Origin + Continent\ of\ Garden\ (G) + Climatic\ Distance + O{:}G + O{:}D + D{:}G + O{:}D{:}G + (1|Population) \quad (3)$$

In this model, a 3-way interaction between continent of origin, continent of garden, and climatic distance may indicate that the strength of local adaptation varies based not only on the range from which populations originated but also the range in which they are being tested. In other words, this model aims to test whether locally adapted European populations maintain signatures of local adaptation in North America and vice versa.

The roles of cyanogenesis and herbivory in local adaptation

The presence of cyanogenesis clines across latitude on both continents was determined separately for *Ac/ac* and *Li/li* by assessing the proportion of plants in each population containing at least one dominant *Ac* or *Li* allele using the following model structure:

$$Proportion\ \mathrm{Ac}\ or\ \mathrm{Li}\ Plants \sim Latitude + Continent\ of\ Origin + Latitude{:}Continent\ of\ Origin + (1|Population) \quad (7)$$

An interaction between latitude and continent of origin would indicate a difference in the slope of clines in the presence of *Ac* and/or *Li* based on the range in which those clines are occurring (native European or introduced North American).

The effect of cyanotype (AcLi, Acli, acLi, or acli) on local adaptation (Q3) was assessed for all eight response variables using the following model structure:

$$Response \sim Cyanotype\ (C) + Garden\ Location\ (L) + Climatic\ Distance\ (D) + C{:}L + C{:}D + C{:}L{:}D + (1|Population) \quad (8)$$

An interaction between cyanotype and climatic distance would indicate that cyanogenesis or its metabolic components contribute to the strength of local adaptation in *T. repens*. A 3-way interaction between cyanotype, garden location, and climatic distance would indicate that differential selection for or against a given cyanotype in a particular common environment impacts the strength of local adaptation in that environment. This might be expected due to

consistently observed latitudinal cyanogenesis clines across multiple types of environmental gradients in *T. repens*.

Bioclimatic variables as individual drivers of local adaptation

We also measured associations between the seven response variables and each of the six bioclimatic variables of interest individually by determining the difference in z-score standardized values for each variable between the location of each population of origin and each common garden location using the following model structure:

$$Response \sim Bioclimatic\ Variable + \ Bioclimatic\ Variable^2 + (1|Population) \qquad (4)$$

For this set of analyses, all common gardens were analyzed separately, and all quantitative (non-binomial) response variables were z-score standardized to allow for simultaneous direct comparisons of the relative strength of linear and quadratic predictor variables. Within each garden, we were therefore able to directly compare *P* values across all bioclimatic variables for both linear and quadratic fits (**Tables S3-S6**). For each response variable, we selected the most influential bioclimatic variable (i.e., the lowest *P*, but only when at least one *P* value was significant at a level of $P < 0.1$, highlighted in red in **Tables S3-S6**). Across all response variables in each garden, we then calculated the percentage of these most influential bioclimatic variables that were either temperature-based (BIO1, BIO5, or BIO6) or precipitation-based (BIO12, BIO13, and BIO14) and compared these percentages across the full experiment, across latitudes, and across ranges (Q4).

Adaptation lags in response to climate warming

We sought to investigate the presence of adaptation lags using a space-for-time substitution (Q5). We performed an additional analysis using a similar model structure as before, but only using

changes in raw values for MAT (i.e., not z-score standardized, hereafter referred to as ΔMAT) as the bioclimatic variable of interest.

$$Response \sim \Delta MAT + \Delta MAT^2 + (1|Population) \quad (5)$$

If the peak of the quadratic relationship in each model is approximately equal to zero, it would be considered evidence of local adaptation to MAT, because maximum fitness occurs in populations with the same mean annual temperature as the common garden site. If the peak of the quadratic relationship is instead shifted towards populations of warmer origins than are occurring at the common garden site (i.e., these warmer-adapted populations are outperforming the local populations), this would be considered evidence of an adaptation lag. We tested whether the shift of peaks away from a ΔMAT of zero and towards populations of warmer origins were significant by also fitting a similar model to above, but excluding the linear predictor:

$$Response \sim \Delta MAT^2 + (1|Population) \quad (6)$$

This additional model constrains the peak of the distribution to be centred around a ΔMAT of zero (approximating the outcome of local adaptation as opposed to an adaptation lag). We then compared the models with and without the linear predictor using the *anova* and *AIC* functions in R, with P values < 0.05 (along with ΔAIC values < 0) indicating a significant shift of the peak.

We focused our interpretation of this analysis based solely on the North American populations in the Mississauga common garden because the sampling design of North American populations along a transect and the location of the Mississauga garden within that sampled transect provide the most appropriate subset of the full experiment data to detect spatial lags. We include the results for European populations within the Uppsala common garden in **Table S7** for completeness, but the Uppsala common garden did not exhibit any trend in fitness based on climatic distance, precluding the possibility of an adaptation lag being detected. The Lafayette

and Montpellier common gardens were excluded from this analysis because they are near the southern extremes of our sampled populations, precluding the ability to detect adaptation lags.

Results

Q1: Are T. repens populations locally adapted to their climate of origin and does the strength of local adaptation differ across space?

We found strong evidence of local adaptation, and the strength of local adaptation in *T. repens* varied among gardens for multiple measures of fitness. Climatic distance between each population of origin and the common garden locations strongly predicted all measures of sexual fitness and survival (**Table 1**). In general, populations tended to have higher fitness with lower climatic distance, indicating that populations of more similar climatic origins to the climate of each common garden were better adapted. The only response variables not showing evidence of local adaptation were the measures of vegetative growth used as proxies for clonal fitness (i.e., maximum plant area and growth rate). The interaction of common garden location and climatic distance affected the number of flower heads and seed set mass, but not other measures of fitness or herbivory (**Table 1**; **Figure S3**). In general, the strength of local adaptation, measured as the slope of the relationship between sexual fitness and climatic distance, was greater in the more southern common garden location on each continent (**Figure 2**). For the number of flower heads, the slope for Lafayette was 3.4× steeper than the slope for Mississauga and the slope for Montpellier was 349× steeper than the slope for Uppsala. Similarly, for seed set mass, the slope for Lafayette was 2.3× steeper than the slope for Mississauga and the slope for Montpellier was 384× steeper than the slope for Uppsala. The main effect of garden location affected all measures of fitness and herbivory, which reflects the large differences in environment between the

gardens. By contrast, the main effect of continent of origin and its interactions with garden location and/or climatic distance did not affect any measure of fitness or herbivory (**Table 1**).

Q2: Does T. repens exhibit stronger local adaptation in its native range than in its introduced range?

We observed strong local adaptation in both the native and introduced ranges of *T. repens*. Continent of origin, continent of the common garden, and climatic distance all interacted to affect all quantitative measures of sexual fitness, survival, and growth rate of *T. repens* (**Table 2**). Specifically, populations only exhibited strong signatures of local adaptation when they were transplanted to common gardens on the same continent from which they originated (**Figure 3**). For number of flower heads, in common gardens located in the native range (Europe), the slope for European *T. repens* populations was 12× steeper than the slope for North American populations. In common gardens located in the introduced range (North America), the slope for North American *T. repens* populations was 20× steeper than the slope for European populations. Similarly, for seed set mass, in common gardens located in Europe, the slope for European *T. repens* populations was 24× steeper than the slope for North American populations. In common gardens located in North America, the slope for North American *T. repens* populations was 7.8× steeper than the slope for European populations. The main effect of climatic distance affected whether or not plants flowered and produced seeds, but climatic distance did not interact with the continent of origin or the continent of the common garden to affect these components of fitness (**Table 2**; **Figure S4**). However, climatic distance and continent of origin did interact to affect maximum plant area and herbivory. Together across all gardens, plants from European origins that were transplanted shorter climatic distances grew larger, despite experiencing greater levels

of herbivory, while size and herbivory for plants from North American origins were not significantly affected by climatic distance overall (**Table 2**; **Figure S4**).

Q3: What are the roles of cyanogenesis and herbivory in driving local adaptation across space?

Both *Ac/ac* and *Li/li* exhibited strong latitudinal gene frequency clines among *T. repens* populations from both continents (**Table S8**). However, the lack of an interaction between latitude and continent of origin for both loci indicates no differences in the steepness of cyanogenesis clines between the native and introduced ranges of *T. repens* (**Figure S5**). In the full experiment, the main effect of cyanotype affected survival, whether or not plants produced flowers and/or seeds, and maximum plant area, with cyanogenic plants generally exhibiting greater fitness than acyanogenic plants, regardless of the environment in which the common garden was found (**Table S9**). However, cyanotype alone did not affect the level of herbivory occurring on *T. repens* plants, and cyanotype did not interact with either common garden location or climatic distance to affect any measures of fitness or herbivory (**Table S9**).

Q4: What bioclimatic variables have the strongest impact on fitness across space in T. repens?

Climatic variables related to both temperature and precipitation predicted variation in fitness in *T. repens*, but the most influential predictors varied between garden locations, with patterns emerging based on latitude (**Tables S3-S6**, red highlights). For the full experiment, 58% of the most influential predictors were temperature-based (mean annual temperature, maximum temperature of the warmest month, or minimum temperature of the coldest month), while 42% were precipitation-based (annual precipitation, precipitation of the wettest month, or precipitation of the driest month). In the more southern gardens on each continent (Lafayette and Montpellier), 67% of the most influential predictors were precipitation-based, while only 33% were temperature-based. Conversely, in the more northern gardens on each continent

(Mississauga and Uppsala), 80% of the most influential predictors were temperature-based, while only 20% of were precipitation-based. We did not find evidence that the native and introduced ranges of *T. repens* were differentially affected by temperature or precipitation variables. In the native range (Uppsala and Montpellier, combined), 57% of the most influential predictors were temperature-based and 43% were precipitation-based. In the introduced range (Mississauga and Lafayette, combined), 58% of the most influential predictors were temperature-based, while 42% were precipitation-based.

Q5: Are there adaptation lags to climate change in T. repens?

North American populations of *T. repens* demonstrated evidence of an adaptation lag in the northern garden in the introduced range (**Table 3**). Survival, seed set mass, and maximum plant area all exhibited a significant quadratic relationship with ΔMAT between locations of origin and the Mississauga common garden (**Figure 4**). A quadratic relationship centred on a ΔMAT of zero would represent local adaptation, while a relationship centred on a ΔMAT > 0 would represent an adaptation lag. For the proportion of plants survived per population, the peak of the quadratic relationship experienced a significant shift to a climate of origin with a MAT 3.4°C greater than Mississauga ($\chi^2_{1,4}$ = 4.159, P = 0.041, ΔAIC = −2.159), with the predicted maximum survival being 5.3% greater than at a ΔMAT of zero. For mean seed set mass per population, the peak of the quadratic relationship occurred at a MAT of 2.0°C greater than Mississauga, with the predicted maximum seed set mass being 5.2% greater than at a ΔMAT of zero. However, this shift was not statistically significant ($\chi^2_{1,5}$ = 0.640, P = 0.423, ΔAIC = 1.360). For mean maximum plant area per population, the peak of the quadratic relationship experienced a shift to a climate of origin with a MAT of 2.1°C ($\chi^2_{1,5}$ = 3.721, P = 0.054, ΔAIC = −1.721) greater than Mississauga, with the predicted maximum plant area being 3.9% greater than at a ΔMAT of zero.

Along our transect of sampled populations, a shift in the peak of the quadratic relationship towards populations 2.0-3.1°C warmer than the Mississauga common garden represents a 2.1-3.2° shift in latitude towards the equator. Additionally, whether or not plants flowered or produced seeds increased linearly with ΔMAT, indicating plants of warmer origins were more likely to flower and produce seeds (**Figure 4**), while the number of flower heads and growth rate did not exhibit any significant linear or quadratic trends with ΔMAT (**Table 3**).

Discussion

The aim of our study was to provide insight into the potential for rapid local adaptation in introduced plant populations in response to novel environments across space and time. The results of our transcontinental experiment in the native (Europe) and introduced (North America) ranges of *T. repens* lead to two main conclusions: 1) rapid local adaptation to climatic variation across space has contributed to the ecological success of *T. repens* in its introduced range; 2) climate warming has resulted in some evidence of a weakening of local adaptation over time in the introduced range of *T. repens.* Our conclusions are based on five experimental results. First, *T. repens* exhibited local adaptation to climate overall, with greater fitness advantages for local populations at lower latitudes (Q1). Second, in European common gardens, only native European populations exhibited local adaptation, while in North American common gardens, only introduced North American populations exhibited local adaptation (Q2). Third, cyanogenesis did not differentially affect the fitness of *T. repens* in different environments (Q3). Fourth, both temperature- and precipitation-based bioclimatic variables predicted fitness, but temperature was a stronger determinant of fitness at higher latitudes, while precipitation was a stronger determinant of fitness at lower latitudes (Q4). Fifth, there is some evidence consistent with an

adaptation lag in response to climate warming, but the signature was weak, and this pattern was only clear in the northern common garden of the introduced range (Q5). Below we discuss our results in the context of the role of rapid local adaptation in the success of biological invasions and how introduced populations may (or may not) be able to adapt to future climate change.

Strength of local adaptation is dependent on latitude

Spatial variation in biotic and abiotic factors across a species' range could affect its ability to adapt to different environments within that range. We found that *T. repens* exhibited significant local adaptation to the abiotic environment based on a measure of climatic distance that encompassed mean annual temperature and precipitation along with annual temperature and precipitation extremes. However, local adaptation differed significantly in strength depending on the latitude of common gardens. Specifically, local adaptation was stronger at lower latitudes in both the native and introduced ranges of *T. repens* when fitness was measured as sexual reproductive success (i.e., number of flower heads and seed set mass). Our results are consistent with transplant experiments using other plant species, which found sexual fitness advantages for local versus foreign plants to be more pronounced at lower latitudes (Colautti and Barrett, 2013; Toräng et al., 2015). Alternatively, life history trade-offs across large latitudinal ranges could result in differences in the observed strength of fitness advantages, depending on the measure of fitness involved (Li et al., 2015; Bastias et al., 2024). In fact, life history trade-offs in *T. repens* specifically have been shown to contribute to local adaptation across divergent environments, with southern populations investing more into sexual reproduction and northern populations investing more into vegetative growth (Wright et al., 2021). If warmer climates are generally more conducive to sexual reproduction than cooler climates, this may explain why sexual fitness

advantages for local populations were more pronounced in Lafayette and Montpellier in our study, while climatic distance had more limited effects on vegetative growth.

Local adaptation in native and introduced ranges

Invasive species have the potential to become more widespread and persistent by rapidly adapting to novel environments. For example, latitudinal cyanogenesis clines in introduced North American *T. repens* populations formed due to rapid local adaptation and sorting of existing genotypes post-introduction (Kooyers and Olsen, 2012; Kooyers and Olsen, 2013; Olsen et al., 2021). We found that populations of *T. repens* only exhibited strong signatures of local adaptation to climate when transplanted into common gardens within the same range as they originated. The observed strong local fitness advantages for North American populations within the North American gardens are therefore indicative of rapid local adaptation of *T. repens* within the past 400 years since its introduction to North America. Selection has been shown to be particularly strong for introduced populations, setting a precedent for rapid local adaptation among invasive species on even shorter timescales than exhibited by *T. repens* (Colautti and Lau, 2015; Simón-Porcar et al., 2021). A meta-analysis by Oduor et al. (2016) found that invasive populations exhibited local adaptation just as frequently as native populations, and that the strength of local adaptation was not affected by time since introduction (ranging from as little as 20 to as many as 500 years). A reciprocal transplant experiment with *Lythrum salicaria* indicated local adaptation to all three common garden sites spanning a 10° latitudinal range, which occurred in ~200 years since its introduction to eastern North America (Colautti and Barrett, 2013). Even faster still is adaptive evolution in the form of trait divergence between coastal and inland environments in *Eschscholzia californica* in its introduced range, which has occurred in the past 150 years (Leger and Rice, 2007). Similarly, clines in flowering phenology of invasive

Hypericum canariense have evolved within only 50 years (Dlugosch and Parker, 2008). Taken together, our results strengthen the mounting evidence that rapid local adaptation to climate plays an important role in the ecological success of invasive species in their new range.

Interestingly, *T. repens* populations from the introduced range did not exhibit signatures of local adaptation to similar climates when transplanted back into their native range. Similarly, native populations did not maintain their strong signatures of local adaptation to climate when transplanted into the introduced range. These findings demonstrate a clear cost of rapid adaptation, with local fitness advantages not extending across continents, even when accounting for similarity among climates. Explanations for this lack of maintenance of local adaptation across continents could be due to unmeasured biotic and abiotic aspects of the environment.

Abiotic explanations for the lack of local adaptation across continents could involve shifts in photoperiod or precipitation regimes between Europe and North America due to similar climates on the two continents being associated with different latitudes. In general, European environments have longer day lengths in summer and shorter day lengths in winter than those of similar climates in North America, with differences being more pronounced at progressively higher latitudes. This photoperiodic shift between the continents could have affected the initiation of flowering and/or the timing of peak flowering for populations that were transplanted across continents, leading to differences in the strength of local adaptation. Halbritter et al. (2015) found that local adaptation was still evident in reciprocal transplants conducted between high elevation and high latitude *Plantago* populations with similar climates, allowing for speculation of a role of photoperiod in affecting fitness. Additionally, populations of an obligate long-day plant, *Mimulus guttatus*, exhibit clinal variation in the critical photoperiod required to initiate flowering, potentially indicating a role of photoperiod in driving local adaptation

(Kooyers et al., 2015). Saikkonen et al. (2012) speculated that the transition to less extreme yearly variation in photoperiod at lower latitudes might facilitate greater invasion success of European species introduced to North America than vice versa, which could be especially true of a facultative long-day plant like *T. repens* (Beatty, 1956). However, our results do not provide evidence of increased success of European populations in North American gardens compared to that of North American populations in European gardens. Additionally, although we accounted for mean annual precipitation and yearly precipitation extremes in our study, these variables do not fully capture the exact timing and volume of precipitation, and the resulting hydrological environment experienced by *T. repens* plants in Europe versus North America. For example, in the Montpellier garden in southern Europe, plants will experience a Mediterranean climate that is not only drier but also more seasonally variable, while in the Lafayette garden in southern North America, plants will experience a sub-tropical environment that is wetter with more limited seasonal variation. This shift in precipitation availability has caused introduced *T. repens* populations to evolve greater drought avoidance as opposed to the more desiccation tolerant strategy observed in Mediterranean populations (Hendrickson et al., 2024).

The biotic environment could also partially explain the lack of local adaptation across continents based on climatic distance (Hargreaves et al, 2020). *Trifolium repens* is a legume that forms mutualistic relationships with N-fixing bacteria in root nodules. Murray-Stoker and Johnson (2024) have demonstrated the existence of locally adapted populations in the *T. repens*-*Rhizobia* mutualism, indicating that local adaptation to biotic factors is possible across environments in this system. Furthermore, in line with classic hypotheses in invasion biology, herbivory could exhibit substantial differences across environments based on expected shifts in herbivore communities in different climates or between continents, which could facilitate success

of a plant species post-introduction (Coley et al., 1985; Blossey and Nötzold, 1995; Keane and Crawley, 2002). If herbivory was likely to explain our results of strong local adaptation within continents but not across continents, we might have expected to see signatures of local adaptation based on the presence of chemical defenses in *T. repens* plants.

Cyanogenesis did not affect local adaptation in either the native or introduced range

We tested whether the presence of latitudinal clines in plant defense indicates a role of defense mechanisms in driving local adaptation in *T. repens*. Among our sampled populations, we demonstrated rapid evolution of latitudinal clines in the presence of both *Ac* and *Li* alleles in the introduced range of *T. repens*, based on their similar strength to clines in the native range. Contrary to our results, all previous work has indicated weaker clines in the introduced range than the native range (Daday, 1954a; Daday, 1958; Innes et al., 2022). Interestingly, our results also indicates that neither cyanogenesis nor the presence/absence of cyanogenic glucosides or linamarase exhibited differential effects on fitness across our common gardens. In fact, local plants exhibited signatures of local adaptation despite experiencing similar or greater levels of herbivory than populations transplanted from further climatic distances. Our study therefore does not demonstrate a role of cyanogenesis or its metabolic components, nor a role of herbivory, in driving local adaptation of *T. repens*, with no evidence of interactions between cyanotype and climatic distance in influencing fitness. Previous studies on local adaptation in *T. repens* found that HCN, cyanogenic glucosides, and linamarase only had marginal, if any, effects on fitness and local adaptation (Wright et al., 2018; Wright et al., 2021). Further, *T. repens* has not consistently experienced changes in cyanogenesis clines in response to recent climate change, which may indicate only marginal involvement of cyanogenesis and/or its metabolic components in local adaptation (Innes et al., 2022). Instead, divergence in life history strategies in response to

climatic factors have been identified as stronger drivers of local adaptation (Wright et al., 2018; Wright et al., 2021), which is consistent with our results.

Our findings contrast with previous work that has speculated on the involvement of trade-offs in the costs and benefits of cyanogenesis in different environments. Namely, previous studies have hypothesized ecological costs of cyanogenesis in cooler climates due to freezing autotoxicity (Daday, 1954a; Daday, 1965) or exacerbated allocation costs of producing the metabolic components of HCN under freezing conditions (Kooyers et al., 2018; but see Fadoul et al., 2023) as possible explanations for low frequencies of cyanogenesis in cooler climates. In our study, cyanogenic plants often demonstrated a fitness advantage, even in Mississauga, which does experience freezing temperatures for much of the year. This finding indicates that even in cooler climates, the benefits of producing HCN as an anti-herbivore defense could outweigh the costs of its production. However, despite overall effects of cyanotype on fitness in our common gardens, we did not find evidence for cyanotype as deterrent to herbivory or as a driver of local adaptation in *T. repens*. In fact, cyanotype did not affect the level of herbivory occurring on *T. repens* plants, indicating that greater prevalence of cyanogenesis in warmer climates is likely not based on an increased ability to defend against greater herbivore pressure.

Alternatively, there is evidence that the ability to produce cyanogenic glucosides and/or linamarase could be advantageous in periods of drought through the storage and recycling of nitrogen (Møller, 2010; Kooyers et al., 2014; Machingura et al., 2016). The first growing season in Mississauga was uncharacteristically dry, resulting in the need for supplemental watering of the common garden to avoid mortality of the full experiment. It is possible that the dry conditions may have also contributed to a fitness advantage for *Ac* and/or *Li* plants. However, any advantages of particular cyanotypes within a given common garden environment did not

confer higher fitness to local plants as opposed to foreign plants based on our measure of climatic distance. Furthermore, we were unable to harvest enough tissue to determine the cyanotype of most of the plants in the study due to concerns over effects of tissue harvest on plant fitness and/or mortality, so the strength of our conclusions with respect to the role of cyanogenesis in adaptation are limited by the relatively low sample size for all statistical analyses involving cyanotype. Further field studies are needed to more explicitly test the costs and benefits of HCN, cyanogenic glucosides, and linamarase, and their effects on local adaptation to climate in *T. repens* across large environmental gradients.

Spatial variation in environmental drivers of local adaptation

While *T. repens* exhibits local adaptation in its native and introduced ranges based on an overall measure of climatic distance, populations may spatially vary in the individual bioclimatic variables responsible for local adaptation. Our results across both ranges demonstrate that at higher latitudes, where local adaptation is weaker, temperature-based variables are stronger determinants of fitness, while at lower latitudes, where local adaptation is stronger, precipitation-based variables are stronger determinants of fitness. Therefore, it is possible that *T. repens* adapts more readily to local precipitation regimes than local temperature regimes, resulting in stronger signatures of local adaptation in lower latitude common gardens, consistent between the native and introduced ranges. This result is supported by a meta-analysis by Siepielski et al. (2017), which found both precipitation and potential evapotranspiration to be stronger predictors of selection than temperature across numerous terrestrial taxa. Alternatively, our results could be affected by the specific environments chosen for the common gardens. In our study, the climates in Lafayette and Montpellier are among the warmest that occur in the range of *T. repens*, and therefore could have been especially difficult to tolerate for populations transplanted from cooler

climates. In turn, Uppsala was somewhat isolated, both geographically and climatically, from the sampled *T. repens* populations, which could have affected our ability to detect local adaptation (or maladaptation) in that environment. Lastly, it is possible that recent climate warming may have already disrupted the ability of local populations to outperform foreign populations, thereby impacting the strength of local adaptation in certain locations.

Evidence of adaptation lags in response to climate warming

The ability of populations to adapt to recent rapid climate warming over time is an important mechanism by which species can mitigate the risk of local extinction and other downstream ecological consequences. Previous studies have demonstrated the incidence of adaptation lags, whereby populations originating from slightly warmer climates would outperform populations with very similar climatic origins to the common garden location (Wilczek et al., 2014; Aguirre-Liguori et al., 2019; Anderson and Wadgymar, 2020). These studies indicate the potential for a disruption in local adaptation, and even the possibility of maladaptation, due to climate warming that is occurring faster than local populations are able to adapt.

In our northern common garden in North America (Mississauga), populations with slightly warmer origins (2-3°C in MAT) outperformed local populations in survival and biomass production (i.e., a proxy for vegetative reproduction) but not measures of sexual fitness. This finding may signal the presence of an adaptation lag and may also provide a partial explanation for the observed weaker local adaptation in Mississauga compared to Lafayette. However, the generalizability of our results should be considered carefully for a few reasons. First, contrary to our expectations, we provide no evidence of either local adaptation or maladaptation in Uppsala. In a large-scale common garden experiment with European populations of *Arabidopsis thaliana*, Wilczek et al. (2014) found that adaptation lags were more pronounced in higher latitude

common garden locations, which was not the case in our study. It would also be pertinent to be able to test for spatial lags in the native range of *T. repens* to which we could directly compare spatial lags in the introduced range. Second, we tested for the presence of adaptation lags based solely on MAT, and Mississauga was the common garden location where MAT most consistently affected plant fitness compared to other bioclimatic variables. Fitness in lower latitude common gardens was instead more heavily influenced by precipitation-based variables, which may not be changing as consistently as are temperature-based variables due to anthropogenic activity. It is therefore possible that adaptation lags to changing environments may not occur consistently across different geographic locations, based on the specific biotic and abiotic factors that most strongly impact local adaptation in those environments. Third, the MAT at the location of the Mississauga common garden in 2020 and 2021 was 1.6°C and 2.0°C warmer, respectively, than the average from the historical time period (1970-2000) used to calculate climatic distances in this study (data extracted from ClimateNA; Wang et al., 2016). Therefore, it may not be unexpected to see populations from slightly warmer origins outperform local populations. The adaptation lag for survival does extend to a peak 3.4°C warmer than Mississauga, and the proportion of plants producing flowers and/or seeds increased linearly with ΔMAT, which still provides evidence for warm-adapted populations having an advantage that exceeds expectations based on the MAT during the years of the experiment. However, across our entire study, we are unable to make broad conclusions about future vulnerability of populations based on the rate of environmental change exceeding the rate of adaptation. Instead, we conclude that the fine-tuned nature of local adaptation (in terms of variation in strength, prominent driving factors, and the potential for maladaptation) to climate in *T. repens* highlights the multifaceted nature of rapid evolution in response to changing environments.

Limitations

While our study offers strong tests of local adaptation to both biotic and abiotic factors in the native and introduced ranges of a cosmopolitan plant, there were limitations that should be considered when interpreting our findings. In particular, we are unable to determine the relative strength of soil conditions and mutualistic interactions compared to climate as drivers of local adaptation. Planting *T. repens* individuals from each population along with the natural soil and microbial communities from their location of origin was infeasible considering our study design and governmental regulations on importation of soil and bacteria. Instead, the best alternative was to maintain consistent treatment of all individuals in all gardens, with *T. repens* individuals planted into the natural soil at each common garden site. It is important to recognize that local edaphic factors have the potential to provide fitness advantages in *T. repens*, as has been demonstrated in other systems (Macel et al., 2007). It is also possible that mutualistic biotic interactions between *T. repens* and nitrogen-fixing bacteria could be involved in providing fitness advantages to local populations (Murray-Stoker and Johnson, 2024). Despite these limitations, signatures of local adaptation to climate were still evident across multiple measures of fitness, strengthening our confidence in the observed patterns.

Conclusions

Our study provides insight into the ability of introduced populations of a widespread invasive plant to adapt to novel environments across space and over time. Signatures of local adaptation were only evident when populations were transplanted within the same range as they originated, indicating both strong and rapid local adaptation in an introduced range of *T. repens* in less than 400 years since introduction. Interestingly, the strength of local adaptation and the most important environmental predictors of plant fitness differed in the north and south of both the

native and introduced range. Specifically, temperature was the most important driver of local adaptation in northern gardens, whereas precipitation was most important in southern gardens. This result indicates that there may be predictable drivers of local adaptation of invasive populations to novel environments, and that these drivers may vary along broad latitudinal gradients. Despite evidence of rapid adaptation to changing environments across space, the northern garden in the introduced range also provided some evidence of local maladaptation compared to populations with slightly warmer origins, which is characteristic of an adaptation lag. Therefore, while local adaptation has occurred quickly in introduced populations of *T. repens* across space, a lack of rapid adaptation to environmental change over time could eventually result in severe erosion of fitness advantages for local populations in the future. Continuing to further our understanding of the ability of native and introduced populations to rapidly adapt to anthropogenic disturbance in space and time is vital to the integrity and maintenance of biodiversity in the face of future environmental change.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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Tables

Table 1 Results of mixed effects models assessing how continent of origin, common garden location, and climatic distance affected the fitness of *T. repens*.

	Plants Producing Flowers			Plants Producing Seeds			# of Flower Heads			Seed Set Mass		
Predictor	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*
Continent of Origin (O)	0.330	1	0.566	0.218	1	0.641	2.314	1	0.128	1.214	1	0.271
Garden Location (L)	**31.703**	**3**	**< 0.001**	**8.104**	**3**	**0.044**	**252.144**	**3**	**< 0.001**	**40.068**	**3**	**< 0.001**
Climatic Distance (D)	**6.015**	**1**	**0.014**	**6.882**	**1**	**0.009**	**13.580**	**1**	**< 0.001**	**14.240**	**1**	**< 0.001**
O × L	0.998	3	0.802	0.952	3	0.813	1.442	3	0.696	4.085	3	0.252
O × D	0.421	1	0.516	1.113	1	0.291	0.277	1	0.599	0.978	1	0.323
L × D	4.439	3	0.218	4.185	3	0.242	**12.713**	**3**	**0.005**	**8.808**	**3**	**0.032**
O × L × D	6.860	3	0.077	7.631	3	0.054	7.764	3	0.051	4.882	3	0.181

	Survival			Maximum Plant Area			Growth Rate			Herbivory		
Predictor	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*	χ^2	df	*P*
Continent of Origin (O)	2.220	1	0.136	0.939	1	0.332	0.094	1	0.759	0.089	1	0.766
Garden Location (L)	**221.162**	**3**	**< 0.001**	**286.435**	**3**	**< 0.001**	**70.328**	**3**	**< 0.001**	**144.426**	**3**	**< 0.001**
Climatic Distance (D)	**6.427**	**1**	**0.011**	0.584	1	0.445	0.089	1	0.766	1.074	1	0.300
O × L	6.155	3	0.104	6.464	3	0.091	1.212	3	0.750	6.635	3	0.084
O × D	0.142	1	0.707	1.747	1	0.186	0.049	1	0.824	0.010	1	0.919
L × D	4.798	3	0.187	5.610	3	0.132	2.887	3	0.409	2.234	3	0.525
O × L × D	7.288	3	0.063	2.693	3	0.441	3.399	3	0.334	3.127	3	0.372

Note: Significant effects ($P < 0.05$) are listed in bold.

Table 2 Results of mixed effects models assessing how continent of origin, continent of garden, and climatic distance affected the fitness of *T. repens*.

	Plants Producing Flowers		Plants Producing Seeds		# of Flower Heads		Seed Set Mass	
Predictor	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Continent of Origin (O)	2.033	0.154	1.754	0.185	0.514	0.474	0.028	0.867
Continent of Garden (G)	**9.184**	**0.002**	1.295	0.255	0.471	0.493	0.037	0.847
Climatic Distance (D)	**28.963**	**< 0.001**	**17.269**	**< 0.001**	**14.310**	**< 0.001**	**16.190**	**< 0.001**
O × G	0.677	0.411	0.942	0.332	0.957	0.328	0.481	0.488
O × D	2.033	0.154	0.036	0.850	1.214	0.271	2.991	0.084
G × D	3.194	0.074	2.490	0.115	1.412	0.235	0.258	0.612
O × G × D	0.501	0.479	0.026	0.871	**19.603**	**< 0.001**	**9.728**	**0.002**

	Survival		Maximum Plant Area		Growth Rate		Herbivory	
Predictor	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
Continent of Origin (O)	0.106	0.745	0.797	0.372	0.053	0.817	0.196	0.658
Continent of Garden (G)	**41.220**	**< 0.001**	**170.553**	**< 0.001**	1.468	0.226	**21.180**	**< 0.001**
Climatic Distance (D)	**10.236**	**0.001**	**58.341**	**< 0.001**	**18.137**	**< 0.001**	**15.810**	**< 0.001**
O × G	0.000	0.993	2.375	0.123	0.465	0.495	0.669	0.413
O × D	1.534	0.216	**34.173**	**< 0.001**	**5.145**	**0.023**	**23.686**	**< 0.001**
G × D	**13.870**	**< 0.001**	1.784	0.182	2.263	0.133	0.240	0.624
O × G × D	**6.515**	**0.011**	0.678	0.410	**7.812**	**0.005**	2.178	0.140

Note: Significant effects ($P < 0.05$) are listed in bold. All predictors are assessed using one degree of freedom.

Table 3 Results of mixed effects models assessing how change in mean annual temperature (ΔMAT) affected the fitness of North American *T. repens* populations in the Mississauga common garden (linear and quadratic fits).

	Survival		Plants Producing Flowers		Plants Producing Seeds		# of Flower Heads		Seed Set Mass		Maximum Plant Area		Growth Rate	
Predictor	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*	χ^2	*P*
ΔMAT	3.758	0.053	**7.425**	**0.006**	**5.397**	**0.020**	0.255	0.614	0.644	0.422	3.799	0.051	0.002	0.962
ΔMAT^2	**4.313**	**0.038**	**8.077**	**0.004**	**7.609**	**0.006**	2.908	0.088	**8.542**	**0.003**	**6.741**	**0.009**	1.808	0.179

Note: Significant effects ($P < 0.05$) are listed in bold. All predictors are assessed using one degree of freedom.

Figure Captions

Figure 1 Maps showing populations of *T. repens* sampled from the (**A**) introduced range (North America, orange circles) and (**B**) native range (Europe, pink circles), as well as the four common garden locations (green diamonds). Coordinates of common garden locations are 42°32’40” N, 79°39’38” W (Mississauga, Ontario, Canada), 30°18’23” N, 92°00’33” W (Lafayette, Louisiana, USA), 59°49’08” N, 17°38’49” W (Uppsala, Sweden), and 43°38’16” N, 3°51’43” W (Montpellier, France).

Figure 2 The effect of climatic distance and common garden location on fitness of *T. repens*. (**A**) Mean number of flower heads and (**B**) mean seed set mass of *T. repens* populations planted in two common gardens in North America (Lafayette in navy and Mississauga in orange) and two common gardens in Europe (Montpellier in green and Uppsala in magenta). Shaded areas represent 95% confidence intervals around each line, while asterisks represent *P* values of relevant fixed effects of Climatic Distance (D) and its interaction with Continent of Origin (O) and Continent of Garden (G) (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Figure 3 The effect of climatic distance on the mean number of flower heads produced by *T. repens* populations originating from its introduced (North America, orange) and native (Europe, pink) ranges and transplanted into common gardens in the (**A**) introduced and (**B**) native ranges. The effect of climatic distance on the mean seed set mass produced by *T. repens* populations originating from its introduced and native ranges and transplanted into common gardens in the (**C**) introduced and (**D**) native ranges. Shaded areas represent 95% confidence intervals around each line, while asterisks represent P values of < 0.05 for relevant fixed effects of Climatic Distance (D) and its interaction with Continent of Origin (O) and Continent of Garden (G).

Figure 4 The effects of the change in mean annual temperature (ΔMAT) on fitness of North American populations of *T. repens* grown in the Mississauga common garden. (**A**) Proportion of plants survived, (**B**) proportion of plants that produced flowers, (**C**) proportion of plants that produced seeds, (**D**) mean seed set mass, and (**E**) mean maximum plant area per population all exhibited evidence of an adaptation lag in response to climate warming, indicated by a positive linear trend or a shift in the peak of the quadratic curve towards populations with warmer climates of origin (denoted as x-axis values > 0). Shaded areas represent 95% confidence intervals around each line.

Figures

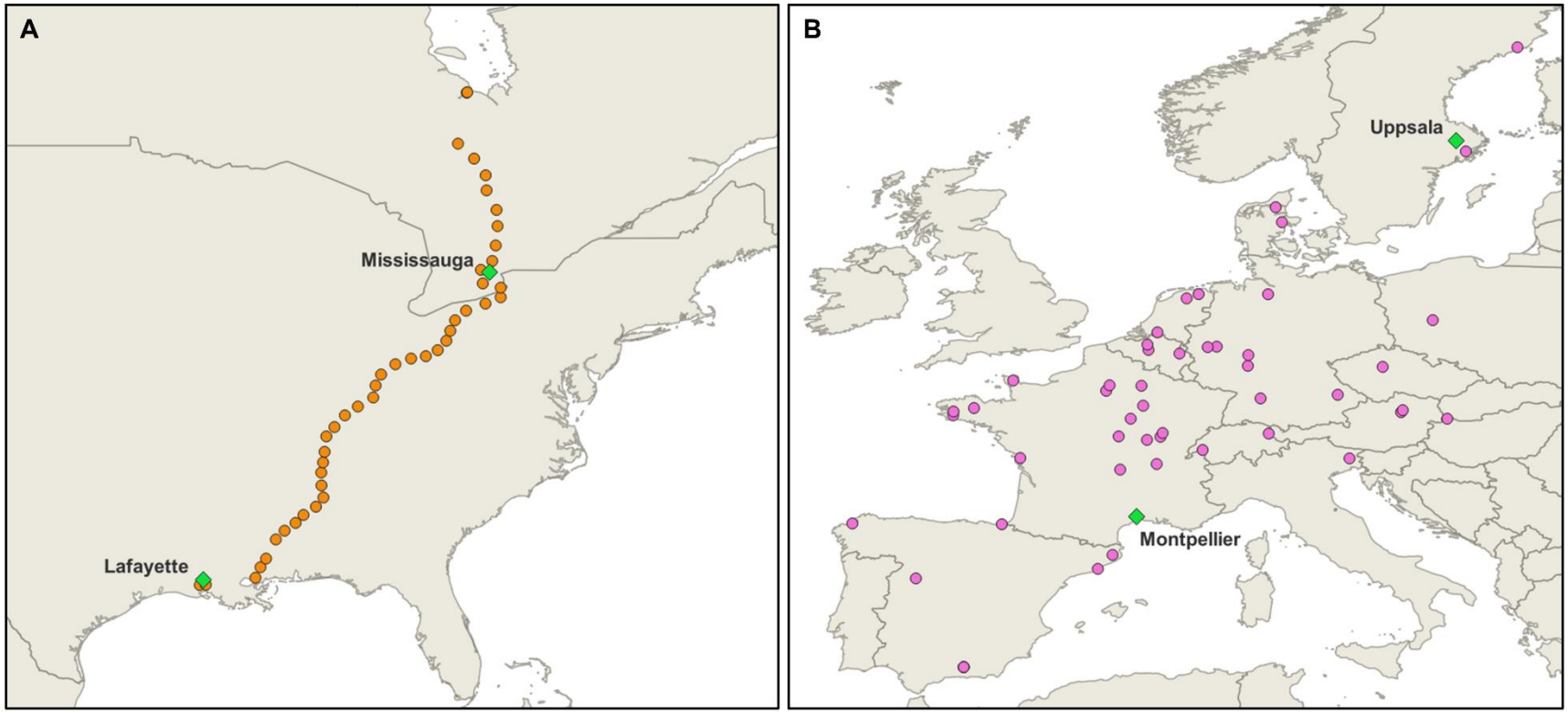

Figure 1

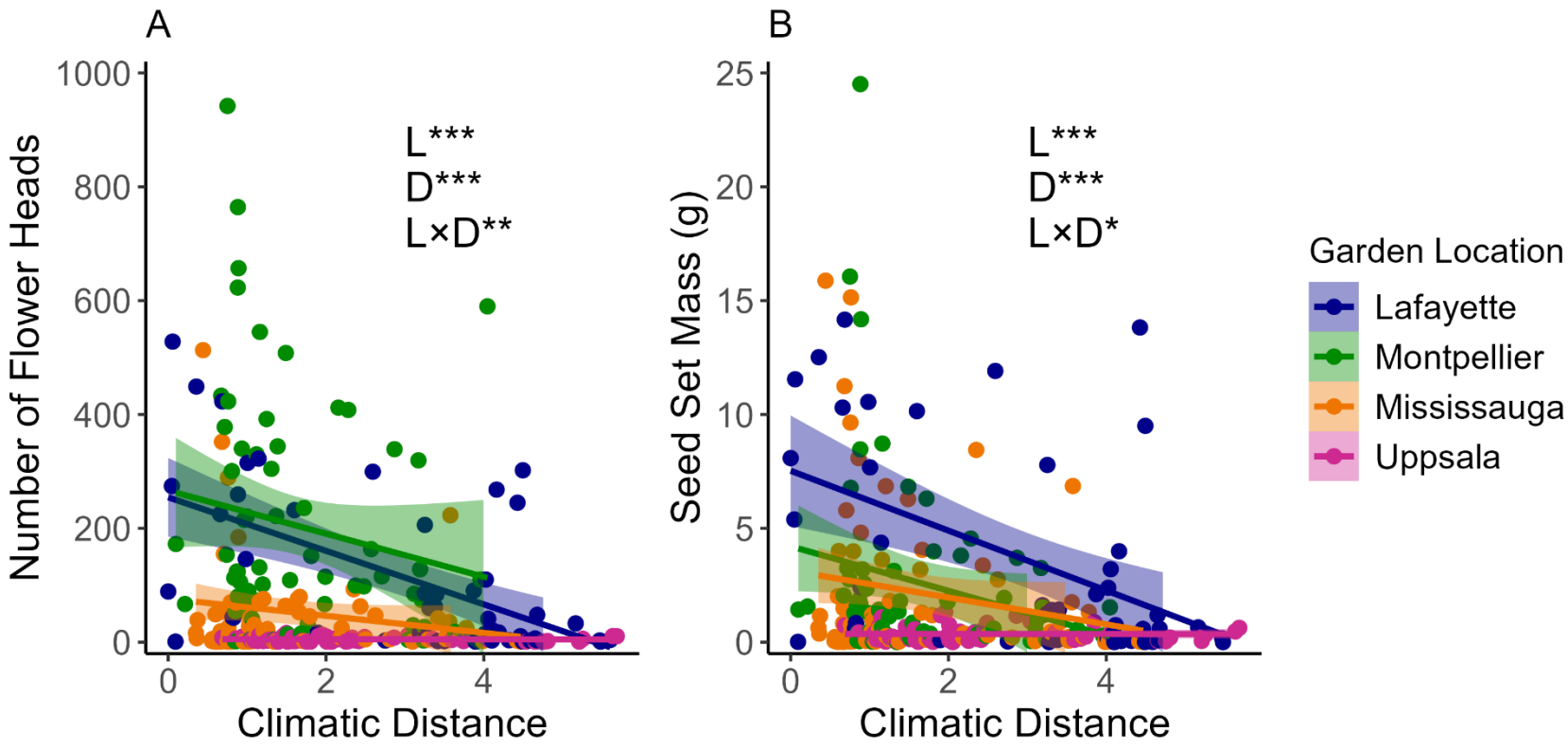
A
B
Number of Flower Heads
Seed Set Mass (g)
Climatic Distance
Climatic Distance
L***
D***
L×D**
L***
D***
L×D*
Garden Location
Lafayette
Montpellier
Mississauga
Uppsala

Figure 2

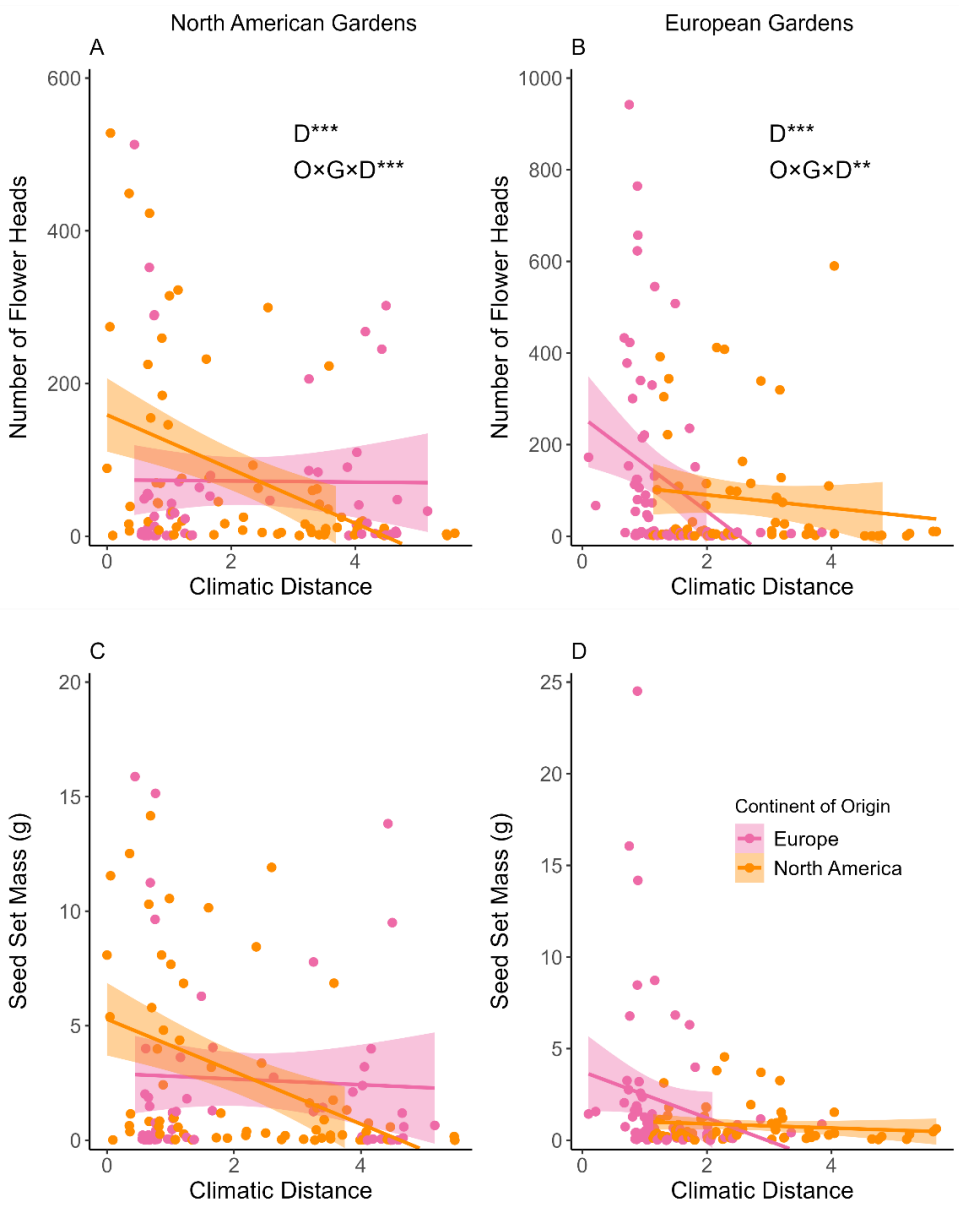
North American Gardens
European Gardens
A
B
C
D
D***
O×G×D***
D***
O×G×D**
Number of Flower Heads
Seed Set Mass (g)
Climatic Distance
Continent of Origin
Europe
North America

Figure 3

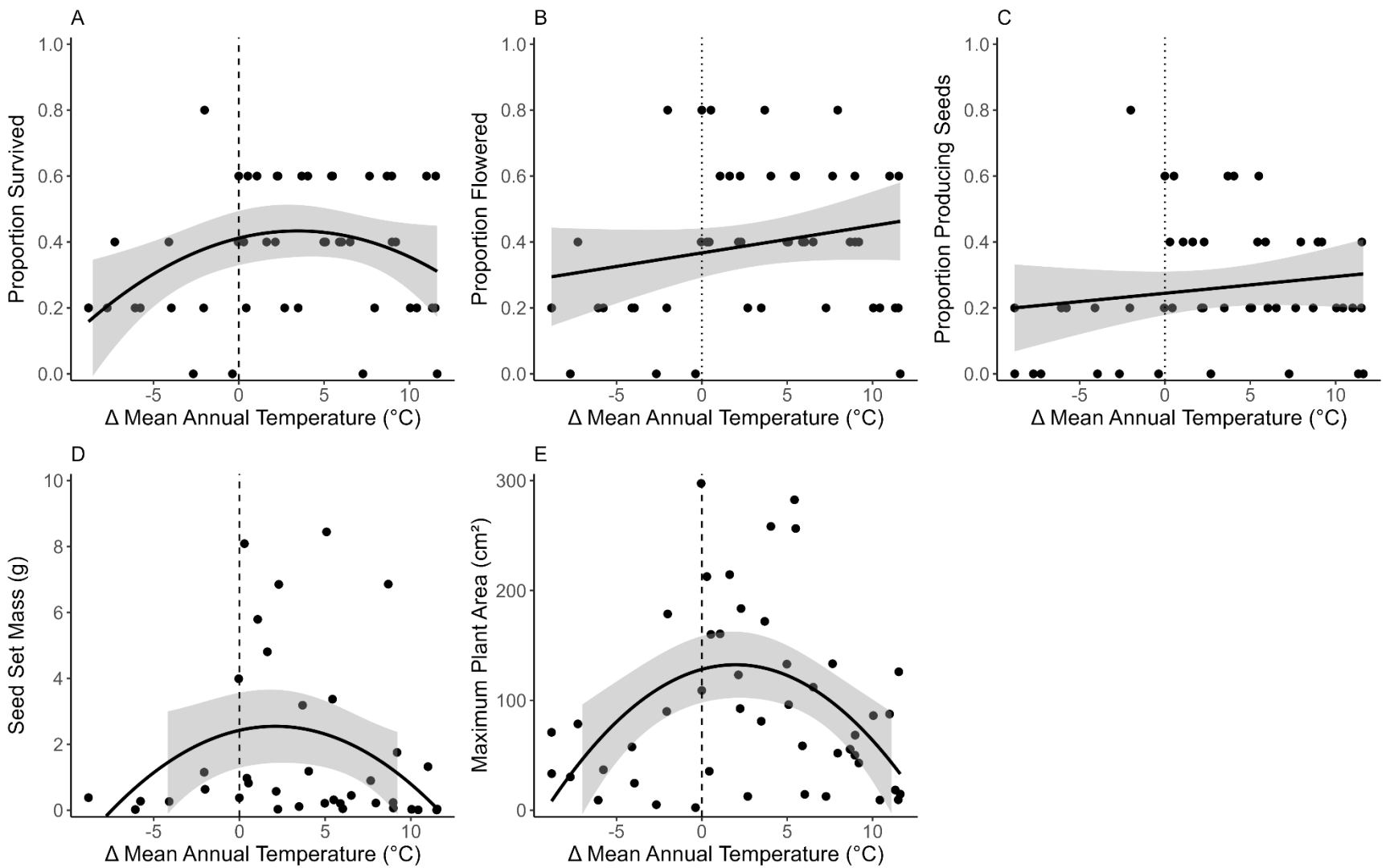
A
B
C
D
E
Proportion Survived
Proportion Flowered
Proportion Producing Seeds
Seed Set Mass (g)
Maximum Plant Area (cm²)
Δ Mean Annual Temperature (°C)

Figure 4