

ORIGINAL ARTICLE

WILEY **MOLECULAR ECOLOGY**

Rapid establishment of a flowering cline in *Medicago polymorpha* after invasion of North America

Emily E. Helliwell¹  | Joshua Faber-Hammond¹ | Zoie C. Lopez¹ | Aaron Garoutte² | Eric von Wettberg³ | Maren L. Friesen^{2,4,5} | Stephanie S. Porter¹¹School of Biological Sciences, Washington State University, Vancouver, Washington²Department of Plant Biology, Michigan State University, East Lansing, Michigan³Department of Plant and Soil Science, The University of Vermont, Burlington, Vermont⁴Department of Plant Pathology, Washington State University, Pullman, Washington⁵Department of Crop and Soil Sciences, Washington State University, Pullman, Washington**Correspondence**

Stephanie S. Porter, School of Biological Sciences, Washington State University, Vancouver, WA.

Email: stephanie.porter@wsu.edu

Funding information

National Science Foundation, Grant/Award Number: DEB-1354878 and DEB-1355216

Abstract

To establish and spread in a new location, an invasive species must be able to carry out its life cycle in novel environmental conditions. A key trait underlying fitness is the shift from vegetative to reproductive growth through floral development. In this study, we used a common garden experiment and genotyping-by-sequencing to test whether the latitudinal flowering cline of the North American invasive plant *Medicago polymorpha* was translocated from its European native range through multiple introductions, or whether the cline rapidly established due to evolution following a genetic bottleneck. Analysis of flowering time in 736 common garden plants showed a latitudinal flowering time cline in both the native and invaded ranges where genotypes from lower latitudes flowered earlier. Genotyping-by-sequencing of 9,658 SNPs in 446 individuals revealed two major subpopulations of *M. polymorpha* in the native range, only one of which is present in the invaded range. Additionally, native range populations have higher genetic diversity than invaded range populations, suggesting that a genetic bottleneck occurred during invasion. All invaded range individuals are closely related to plants collected from native range populations in Portugal and southern Spain, and population assignment tests assigned invaded range individuals to this same narrow source region. Taken together, our results suggest that latitudinal clinal variation in flowering time has rapidly evolved across the invaded range despite a genetic bottleneck following introduction.

KEYWORDS

biological invasions, clines, flowering time, genome wide association study, rapid evolution

1 | INTRODUCTION

A hallmark of a successful biological invasion is the rapid spread of an introduced species across novel environmental conditions it encounters in a new geographical range (Barker, Andonian, Swope, Luster, & Dlugosch, 2017; Bosssdorf et al., 2005; Colautti & Lau, 2015; Prentis, Wilson, Dormontt, Richardson, & Lowe, 2008; Thompson, 1998). Broad-scale geographical gradients in environmental conditions can select for clinal adaptation in phenology, which allows an organism to time the progression of life stages to optimize success under local

seasonal conditions (Donohue, 2017). Rapid formation of a phenotypic latitudinal cline has been demonstrated for various traits in successful invaders, including wing size in *Drosophila subobscura* (Gilchrist, Huey, & Serra, 2001; Huey, Gilchrist, Carlson, Berrigan, & Serra, 2000), plant size in the annual weed *Impatiens glandulifera* (Kollmann & Bañuelos, 2004; flowering time, plant height and biomass, branch and leaf number in *Solidago altissima* (late goldenrod) (Etterson, Toczydlowski, Winkler, Kirschbaum, & McAulay, 2016), plant size in *Eschscholzia californica* (California poppy) (Leger & Rice, 2007), fitness attributes in *Raphanus sativus* (California wild

radish) (Ridley & Ellstrand, 2010) and plant size in yellow starthistle (*Centaurea solstitialis*, Barker et al., 2017). However, despite the importance of the rapid evolution of clinal variation in phenology to biological invasions, few studies have addressed the origins of variation in phenology clines in the invaded range.

For annual plants, the transition from vegetative to reproductive growth requires a large developmental shift that is genetically determined via well-documented molecular pathways and is also cued by environmental factors such as temperature and day-length (Bouché, Woods, & Amasino, 2017; Jung & Müller, 2009; Kazan & Lyons, 2016; Song, Shim, Kinmonth-Schultz, & Imaizumi, 2015). A plant species distributed across a latitudinal gradient can evolve clinal distributions of genotypes that are genetically predisposed to flower at an appropriate time given the local photoperiod and temperature. Latitudinal flowering clines have evolved in several introduced plants, including *Arabidopsis thaliana* (Mouse ear cress, Stinchcombe et al., 2004; Samis et al., 2012), *E. californica* (California poppy, Leger & Rice, 2007) and *Lythrum salicaria* (purple loosestrife, Montague, Barrett, & Eckert, 2008; Colautti & Barrett, 2013). The rapid evolution of clinal variation in phenology has received increased attention because of its importance to the success of invasive species and to the anticipated role such evolution will play for the survival of species in response to changing climatic conditions (Arft et al., 1999; Colautti & Barrett, 2010, 2013; Davis, Shaw, & Etterson, 2005; Skelly et al., 2007; Spicer & Chapman, 1990; Woods, Hastings, Turley, Heard, & Agrawal, 2012). Yet, we have relatively few empirical studies of whether clinal variation in the invaded range is established based on standing variation and/or new mutations present in a founding population seeded by a narrow source from the native range, from multiple introductions from diverse latitudes across the native range that results in a simple transfer of the native range cline, or through introgression from another species as a source of genetic variation postinvasion.

Genetic approaches are necessary to determine the origin of a cline in a species' invaded range. While reduced genetic diversity through founder effects is commonly observed in the invaded range (Dlugosch & Parker, 2008), there are examples in which multiple introductions are observed. For example, multiple introductions of the perennial shrub, *Verbascum thapsus*, to California led to diverse populations adapted to a variety of temperatures (Dlugosch & Parker, 2008), and multiple introductions of black cherry, *Prunus serotina*, from northeastern United States led to increased genetic diversity in Europe (Pairon et al., 2010). However, some invasive plants successfully spread across large geographical ranges despite a single point of origin in the founding populations. For example, founders from a single point of origin led to the successful invasion of *Hypericum canariense*, (St. John's wort), from the Canary Islands to California and Hawaii (Dlugosch & Parker, 2008; Maron, Elmhendorf, & Vilà, 2007), and *C. solstitialis* (Yellow starthistle), from Spain and southern France to Chile, California and the Pacific Northwest United States (Barker et al., 2017). Using genetic markers to determine the geographical origins of an invasive plant species, we can determine whether a phenotypic

cline present in the invaded range has been transported intact from numerous points of origin across a cline that already exists in the native range, or whether a cline has evolved from founders from a single region of origin. There are several mechanisms by which introduced species can adapt to environmental variation across a novel geographical range, such as genetic interactions during admixture, the presence of large effect alleles in founding populations, epigenetic processes and transcriptional variation such as alternative splicing (reviewed in Ellstrand & Schierenbeck, 2000; Dlugosch, Anderson, Braasch, Cang, & Gillette, 2015; Estoup et al., 2016; Patalano et al., 2015; Singh, Börger, More, & Sturmbauer, 2017; Price et al., 2018). Polymorphisms in loci that have regulatory roles would be able to alter a wide range of physiological processes, including those that could underlie clinal variation (Mitchell-Olds & Schmitt, 2006).

Medicago polymorpha is a wild relative of alfalfa (*Medicago sativa*) and the model legume species *Medicago truncatula* (Young et al., 2011), and is a highly selfing annual that occurs in diverse habitats. Medics are native to the Mediterranean region spanning Europe and North Africa (Lesins & Lesins, 1979). *Medicago polymorpha* was introduced to the Americas in the 1700s by Spanish settlers as a forage crop (Westgate, 1908; Wing, 1912; Spira & Wagner, 1983; Porter, Stanton, & Rice, 2011). It is now widespread throughout North and South America and is classified as a prohibited and regulated noxious weed by the United States Department of Agriculture for Arizona, due to its ability to displace resident vegetation and detrimental effects on pasture quality (USDA-NCRS Plant Fact Sheet, https://plants.usda.gov/factsheet/pdf/fs_mepo3.pdf). *Medicago polymorpha* lineages vary substantially in flowering time, which is a factor in its ability to spread along a large geographical range (Ehrman & Cocks, 1996). Analysis of *M. polymorpha* collections from Chile revealed both a latitudinal and longitudinal cline for the onset of flowering time, as well as mean annual precipitation/potential evapotranspiration (PP/ETP) (Del Pozo, Ovalle, Aronson, & Avendaño, 2002). Using both an extensive new collection of populations in the native and invaded ranges with a focus on western Europe and North America along with genotypes from existing biological collections, we asked the following: (a) Is there a latitudinal flowering cline within the native or invaded range of *M. polymorpha*? Upon finding evidence for both clines, we investigated the population genetic context in which the flowering time cline has evolved in the invaded range, and (b) do patterns of genome-wide diversity support a scenario of invasion from a narrow geographical origin and subsequent rapid establishment of a flowering time cline across the geographical extent of the invaded range? Or, do they support a scenario of invasion from multiple origins across the extent of the native range cline into similar latitudes in the invaded range, and thus establishment of a cline in the invaded range due to transport of the cline intact? To answer these questions, we combined a common garden experiment with genotyping-by-sequencing SNP markers to investigate the mechanisms that have resulted in clinal variation for *M. polymorpha* flowering time across a wide latitudinal range in the Americas.

2 | METHODS

2.1 | Collections

Our study of *Medicago polymorpha* across native and invaded ranges included plants grown from seeds collected from the wild as well as from USDA accessions. Wild *M. polymorpha* populations were located using the Global Biodiversity Information Facility website (GBIF, www.gbif.org). Population samples of wild lineages from 29 populations were collected in the native range throughout Europe and from 11 populations in the North American invaded range. Individual USDA *M. polymorpha* seed accession lineages originating from 28 invaded range populations and 47 native range populations were also used, each represented by a single accession. At each wild population, three mature pods from up to 30 plants were collected from across the extent of a patch of *M. polymorpha*. If plants at a site had died back due to summer senescence, 30 individual pods that were each at least 1 m apart directly from the ground at a site were collected with the assumption that each pod represents a unique maternal lineage because plants were typically <20 cm in diameter and *M. polymorpha* pods appear rarely to disperse larger distances (Lesins & Lesins, 1979). Sampling near water bodies or rivers was avoided due to the potential for water-mediated pod transport. All field collections occurred between May and September of 2015.

2.2 | Common garden experiment

The common garden experiment was carried out at Washington State University Vancouver (45.7328054, -122.635967). Three seeds per genotype were planted into 158-ml cylindrical pots, filled with Sungro Sunshine Mix #1 potting soil (Sungro Horticulture, MA, USA), 5 mm below the soil surface. Most seeds originated from wild-collected seeds that had undergone one generation of growth and reproduction in a common garden ($n = 441$) in the Michigan State University greenhouse. However, some seeds were planted directly into the common garden experiment from field-collected pods ($n = 217$). During germination, seeds were mist-irrigated twice a day for 20 min; following seedling emergence, watering was reduced to twice a day for 10 min. Plants were fertilized by placing 10 Osmocote Plus Outdoor & Indoor fertilizer pellets (Scotts Company, Marysville, OH, USA) at the base of each plant, and, 7 days postplanting, all plants were inoculated with 1 ml of a 1:1 mixture of two rhizobium symbiont strains, *Ensifer medicae* WSM419 (Reeve et al., 2010) and *E. meliloti* Rm1021 (Galibert et al. 2001). Prior to inoculation, these strains were cultured separately in tryptone yeast broth shaken at 300 rpm at 30°C. After 2 days of growth, rhizobium cultures were each centrifuged to a soft pellet and resuspended in water to a concentration of 10^6 cells/ml based on OD600. Leaf tissue was collected to provide genomic DNA for the GBS analysis 4 weeks after planting (see below). During this sampling, plants were thinned to one seedling per cone. Plants were checked daily for first flowers, and flowering date was recorded when petals were open at least 45°.

2.3 | Data sets used in analyses

Growth data were collected from 736 common garden plants consisting of 661 samples originating from the wild populations described in the "Collections" section above and 75 USDA samples. These 736 plants are referred to as the greenhouse population, and this data set was used to investigate latitudinal variation in flowering time. For our genotypic analyses, we reference two main data sets: the full GBS population and the deep GBS population. The full GBS population is comprised of the 446 plants from which we extracted genomic DNA for GBS sequencing, from across the 736 *M. polymorpha* greenhouse-grown plants (Supporting Information Table S1). Our full GBS population includes 285 individuals from 71 native range populations and 161 individuals from 38 invaded range populations. Of these individuals, 377 are wild lineages we collected in the field and 69 are USDA accessions. In the native range, there are 1–25 individuals per population with an average population size of 4. In the invaded range, there are 1–15 individuals per population, with an average population size of 4.2. In contrast, the deep GBS population is a subset of the full GBS population that only includes *M. polymorpha* from the wild populations we sampled deeply (i.e., have > five plants per population, $n = 364$; Table 1). The deep GBS population includes 18 native range populations totalling 232 individuals, and 11 invaded range populations totalling 132 individuals. These populations consist of 5–25 individuals, with an average of 12 and 12.8 per population in the invaded native range, respectively.

2.4 | Flowering cline analysis

The presence of a latitudinal flowering cline was tested in the invaded range of *M. polymorpha* using mixed-effects Cox proportional hazards regression in the packages *coxme* and *survival* (Therneau, 2015) in R 3.3.2 (R Core Team, 2016). The number of days until flowering for native range and invaded range plants was modelled by range (native or invaded), latitude of collection site, and the interaction of range and latitude as fixed effects. The model is right censored, with plants that never flowered assigned a 140-day flowering time to signify the end of the observation period. Each *M. polymorpha* genotype is considered an experimental unit, and population and greenhouse rack (position within the greenhouse) are analysed as random effects. The model was run on the full greenhouse population, the full GBS population and the deep GBS population. Invaded range individuals from the Southern Hemisphere were also tested for a latitudinal flowering cline, but this was a low-powered test due to the small sample size of 22 individuals. To determine whether maternal effects drive the trends in the data due to the 29.5% of genotypes that were planted directly into the common garden without a generation of growth in the greenhouse, we ran this model with and without these field-collected seeds. We tested the significance of random effects with the likelihood ratio statistic as the difference in $-2\ln$ between models differing in the inclusion versus exclusion of each random effect. The statistic was compared to a chi-square distribution with one degree of freedom, which is conservative for

TABLE 1 Deep GBS Populations. Deep GBS populations refer to sites which more than 5 individuals of *Medicago polymorpha* were taken. Range, origin in the native or the invaded range of *M. polymorpha*; Population, the specific site sampled within a US state or within a country, from north to south; Site ID, abbreviation for each population; Origin, collection site for each population; N, the number of *M. polymorpha* individuals sampled from a population

Range	Population	Site ID	Origin	Latitude	Longitude	N
Invaded	Arizona	AZ	Casa Grande, AZ, USA	32.87803	-111.749	12
Invaded	California 1	CA1	Arcata, CA, USA	40.87011	-124.113	14
Invaded	California 2	CA2	Napa, CA, USA	38.828	-122.347	14
Invaded	California 3	CA3	Berkeley, CA, USA	37.88042	-122.259	15
Invaded	California 4	CA4	Temecula, CA, USA	33.46594	-117.04	15
Invaded	California 5	CA5	Vista, CA, USA	33.14846	-117.25	13
Invaded	California 6	CA6	San Diego, California, USA	32.772	-117.075	14
Invaded	Florida 1	FL1	Saint Augustine, FL, USA	29.89472	-81.3144	10
Invaded	Florida 2	FL2	Palatka, FL, USA	29.64778	-81.6514	6
Invaded	Florida 3	FL3	Hillsborough, FL, USA	27.91	-82.35	5
Invaded	Oregon	OR	Roseburg, OR, USA	43.23	-123.356	14
Native	France 1	FRA1	Lac du Salagou, France	43.67624	3.352244	14
Native	France 2	FRA2	Arles, France	43.61891	4.813317	18
Native	France 3	FRA3	Frontignan, France	43.47612	3.762642	14
Native	France 4	FRA4	Narbonne, France	43.15515	3.070667	25
Native	Italy 1	ITA1	Breccia, Italy	45.54167	10.21667	11
Native	Italy 2	ITA2	Mirabilandia, Italy	44.33056	12.2955	7
Native	Italy 3	ITA3	San Carlo, Italy	44.0864	12.18779	13
Native	Italy 4	ITA4	Bolsena, Italy	42.64472	11.98583	5
Native	Italy 5	ITA5	Orbetello, Italy	42.44278	11.22472	7
Native	Italy 6	ITA6	Furbara road, Italy	41.986	12.073	8
Native	Portugal 1	POR1	Lisbon, Portugal	38.81822	-9.1429	13
Native	Portugal 2	POR2	Castro Verde, Portugal	37.70849	-8.09929	14
Native	Portugal 3	POR3	Fuseta, Portugal	37.05418	-7.74268	13
Native	Spain 1	SPA1	Santona, Spain	43.45713	-3.45614	14
Native	Spain 2	SPA2	San Sebastian, Spain	43.39133	-1.79217	14
Native	Spain 3	SPA3	Rio Tia Mayor, Spain	43.39083	-4.50991	14
Native	Spain 4	SPA4	Aznalcollar, Seville, Spain	37.53193	-6.28656	14
Native	Spain 5	SPA5	Huelva, Spain	37.26548	-6.85472	14

random effects (Pinheiro and Bates, 2009). The effects of latitude, range and their interaction on the number of days to flower were examined using a type III sums of squares approach in R (*car*; Fox & Weisberg, 2011).

2.5 | Genotyping-by-sequencing

Individual trifoliate leaves were collected from 4-week-old plants in the full and deep GBS populations and placed into 96-well plates containing steel beads in each well, immediately flash-frozen in liquid nitrogen, and stored at -80°C until DNA extraction. The scissors used to cut leaves were wiped clean with ethanol between each plant, and 50–75 mg of leaf tissue was collected from one plant per cone. Tissue was shipped on dry ice to the University of California, Davis, for DNA extraction. Frozen tissue was pulverized with the Qiagen TissueLyser

II, and genomic DNA was extracted with the Qiagen DNeasy 96 Plant kit according to the manufacturer's protocol. DNA concentration was measured via PicoGreen quantitation on a Tecan Plate Reader.

DNA samples were sent to Cornell for Genotype By Sequencing (GBS) library preparation and sequencing (Craig et al., 2008). Samples were digested with restriction enzyme EcoT22I, barcodes were ligated onto each end, and pooled samples were run on the Illumina HiSeq2500. GBS read outputs were preprocessed at WSU-Vancouver using the *FASTX* toolkit v0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/). First, reads were demultiplexed using *fastx_barcode_splitter*. Next, barcodes and adapter sequences were removed with *fastx_trimmer* and *fastx_clipper*, respectively, and reads were filtered to a minimum of 32 bp. Reads containing base-calls with quality scores less than 30 bp anywhere within the read length were removed to conservatively filter out sequencing errors.

Preprocessed GBS reads were analysed using GlibPSs v1.0.2 (Hapke & Thiele, 2016). GlibPSs was selected due to its handling of reverse-complement reads, in/dels and ability to process reads of variable length. In addition, GlibPSs is optimized for processing GBS data without a reference genome, and preliminary analyses showed *M. polymorpha* is highly diverged from the closest sequenced genome, *Medicago truncatula*, with fewer than 50% of GBS sequences aligning. The GlibPSs pipeline was implemented according to developer recommendations: indloc identified loci in individuals, poploc identified loci at the population level, and indpoploc cross-referenced these outputs. Sequence variants were called using the binomial likelihood ratio method (Glaubitz et al., 2014) with an additional filter requiring variants to be represented by a minimum of four reads within individuals. Loci containing putative in/dels and those resembling highly repetitive sequences below a read depth percentile of 0.5 were removed. Loci were selected for downstream analyses if they occurred in at least 95% of individuals (425/446 individuals total), were between 50 and 96 Bp, had no more than two alleles within any individual and had no more than 10 SNPs within a read across all individuals. SNP calls were made on GlibPSs-exported loci using parseSNPs.py (<https://gist.github.com/peterk87/8409706>). Coverage depth (i.e., sequenced read depth) was calculated for the filtered data set as the median of median coverage per individual using only loci passing GlibPSs filters. To evaluate whether particular classes of loci were influential, we conducted a subset of analyses on SNP data sets with and without multi-allelic loci (loci with > two alleles) and loci that showed observed heterozygosity more than 15% higher than expected heterozygosity ($>1.15 \times 2pq$). Loci with excess heterozygosity could result from misaligned paralogs, or deviations from Hardy-Weinberg assumptions such as heterozygote advantage.

2.6 | Population structure analysis

To determine the number of population clusters in *M. polymorpha*, we ran STRUCTURE v2.3.4 (Pritchard, Stephens, & Donnelly, 2000) on two subsets of the data: the deep GBS population of 364 individuals (Table 1), and the full GBS population of 446 individuals (Supporting Information Table S1). In both cases, five iterations of $K = 1$ through six were run, and 10,000 burn-ins with 20,000 replicates were run. The program STRUCTURE HARVESTER was used to determine the optimal number of clusters by use of Evanno's delta K method (Earl & vonHoldt, 2012; Evanno, Regnaut, & Goudet, 2005). To confirm patterns observed by STRUCTURE in this primarily inbreeding species, principal component analysis was also conducted on the deep GBS population (adeqenet; Jombart & Ahmed, 2011).

Summary statistics (H_o , H_s , G_{is} and G_{st}) were calculated for the deep GBS populations (GENODIVE v2.0b23; Meirmans & Van Tienderen, 2004), and confidence intervals were calculated by bootstrapping. Pairwise F_{st} values were calculated for deep GBS populations using Weir and Cockerham's F_{st} estimate (Weir & Cockerham, 1984) (hierFstat; Goudet, 2005). Resulting pairwise F_{st} values were clustered with the average hierarchical clustering method (pvclust; Suzuki &

Shimodaira, 2006)), and a heatmap was generated (heatmap.2; gplots; (Warnes, et al., 2013)). Bootstrap values were calculated using the average method with 1,000 replications, and values above 95 were considered to be significant (pvclust; (Suzuki & Shimodaira, 2006)).

To evaluate patterns of relatedness among individuals, a neighbor-net (Bryant, 2003) was constructed using all 446 individuals from the full GBS population (Splitstree; (Hudson, 1998)). To find where the genetic variance was partitioned in *M. polymorpha*, we ran an AMOVA in the deep GBS population data set at the levels of Range (native or invaded) Population within range, individual within population and within individual. Subsequent AMOVA tests were run separately on the native range and Invaded range individuals from the deeply sampled populations (PoppR; (Kamvar, Tabima, & Grünwald, 2014)).

To determine the best supported native range source population for populations in the invaded range, we used two complementary population origin assignment tests on invaded range individuals. The first, Genodive, assigns individuals based on allelic similarities between invaded range and native range individuals. The second, Smooth and Continuous AssignmentTS assignment tests, (Scat2), assigns individuals back to specific GPS coordinates based on the allelic frequencies and locations of native range individuals. Similar results from both analyses would bolster confidence in our findings. Genodive assignment tests were conducted using a Monte Carlo sampling method, with an alpha value of 0.002 and 100 replicated data sets (GENODIVE v2.0b23, Meirmans & Van Tienderen, 2004). Scat2 (Wasser et al., 2004) used the -A option with the default run-length settings of 100 iterations of the MCMC scheme, 10 steps through the Markov chain for each iteration and 100 burn-in iterations.

2.7 | GWAS analysis of flowering time

In order to build a haplotype map, GlibPSs output was used to produce a hapmap file for use in GAPIT (v20150208, Lipka et al., 2012) using custom PYTHON (v2.7.9) scripts. In order to select GWAS models that best fit model assumptions, we evaluated the Q-Q plots of 108 GWAS models that include different subsets of the data and that used different kinship algorithms and data transformations. The full and deep GBS populations were split into individuals sampled from the native and invaded ranges of *M. polymorpha*, and each range was run separately and as a combined data set, with raw values and log10 and square root transformations of the data as well as with and without nonflowering individuals. Furthermore, we evaluated three kinship algorithms for each of these models. The GWAS analysis was run in GAPIT (v20150208) using the VanRaden kinship algorithm and a SNP minor allele frequency minimum of 2%. GWAS was also run with the kinship.algorithm set to either "EMMA" or "Loiselle" algorithms. The hapmap was adjusted for each analysis to include only the individuals present in each analysis.

Only one set of models produced acceptable Q-Q plots, and for this set of models, SNPs at 0.05 FDR statistical threshold were identified. Of these, SNPs with a minor allele frequency of < 2% were

removed. The remaining significant SNPs were functionally annotated by comparison with the *M. truncatula* genome (v4.0, Tang et al., 2014) using BLASTN (default parameters) available through the JCVI website (<http://blast.jcvi.org/er-blast/index.cgi?project=mtbe>) to extract *M. truncatula* gene names and their annotations.

3 | RESULTS

3.1 | Latitudinal cline in flowering time

Medicago polymorpha shows a latitudinal cline in flowering time in both the native and invaded ranges, with a stronger cline in the native range (Figure 1). Cox proportional hazards mixed-effects regression analyses of flowering time by latitude for all *M. polymorpha* included in the common garden experiment (field-collected and USDA samples) reveal that latitude predicts flowering time ($X^2 = 27.187$, $p = 1.847\text{e-}07$, Table 2) and that flowering time varies more substantially per degree latitude in the native range, as the cline is steeper in the native range ($X^2 = 12.139$, $p = 0.0004937$, Table 2). The flowering time cline appears to be driven by heritable genetic attributes rather than maternal effects because 70.5% of the individuals for which flowering time was assessed in the common garden had undergone

a generation in the greenhouse. Excluding the 29.5% of plants grown directly from field-collected seed did not change the significance of model terms (Supporting Information Table S2). Regression analysis of flowering time with the subset of plants in the GBS analysis also identified all terms as significant (latitude: $X^2 = 21.828$, $p = 2.983\text{e-}06$; range: $X^2 = 10.523$, $p = 0.0011790$; latitude*range: $X^2 = 11.873$, $p = 0.0005696$, Table 2). In an analogous model run on only the deeply sampled populations used in the GBS analysis, all terms were found to significantly influence flowering time (latitude: $X^2 = 17.5893$, $p = 2.741\text{e-}05$; range: $X^2 = 4.3906$, $p = 0.03614$; latitude*range: $X^2 = 4.9703$, $p = 0.02579$, Table 2). Exploratory analysis of a flowering cline in the Southern Hemisphere invaded range showed that there was not a significant flowering cline among these 22 individuals originating from South America, possibly due to the small sample size.

3.2 | GBS statistics

A total of 1,281,296,058 reads were sequenced across 475 individuals. Following FASTX preprocessing steps, we retained a total of 663,615,735 reads (51.8%). GlibPS analysis identified an average of 58,796 loci per individual and 1,156,672 unique loci at the population level. After GlibPSs quality and length filtering steps and selection of informative loci, we were left with a total of 4,098 SNP-containing loci with 15,992 total SNPs across all individuals. Preliminary phylogenetic analysis revealed that 29 plants did not group with the majority of *M. polymorpha* individuals and appeared to represent a separate species (*M. arabica* based on leaf and pod morphology). Therefore, these samples were removed from all greenhouse and GBS analyses. The removed samples accounted for 6,334 SNPs in our data set that were monomorphic in true *M. polymorpha* samples. This left 9,658 informative SNPs for *M. polymorpha* to be used for downstream

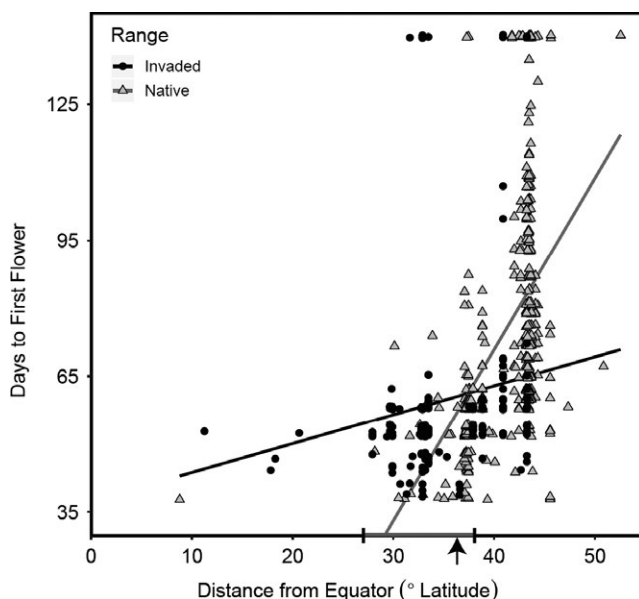


FIGURE 1 Days until first flower across latitudes in native (grey triangles) and invaded (black dots) ranges of *Medicago polymorpha*. Vertical black x-axis tick marks delineate source latitudinal range as identified by Scat2. Cox proportional hazards models generally identified latitude, range and their interactive effect to significantly influence the number of days until flowering, though range was marginally significant within the GBS data set (C , $X^2 = 5.4873$, $df = 2$, p -value = 0.06434, Table 2). Shown are all surviving *M. polymorpha* plants from the WSUV common garden experiment. Of the 736 plants included in the regression analysis, 661 plants originated from field-collected seeds and 75 were obtained from USDA accessions. Black arrow on the x-axis indicates the latitude of Portugal 3, where 95% of invaded range individuals originated from according to Genodive population assignment test

TABLE 2 Analysis of *Medicago polymorpha* flowering time throughout the native and invaded ranges along a latitudinal gradient. Results of Cox proportional hazards model run with all *M. polymorpha* individuals (USDA and field-collected) included in the common garden experiment. Random effects were tested with the likelihood ratio statistic calculated as the difference in $-2\ln$ between models differing in the inclusion versus exclusion of each random effect. The statistic was compared to a chi-square distribution with one degree of freedom. Fixed effects were examined using type III SS ANOVA in R

Fixed effects	Field collection and USDA samples		
	X^2	df	p -Value
Latitude	27.687	1	1.85E-07
Range	11.059	2	0.0009
Latitude*Range	12.139	1	0.0005
Random effects			
Greenhouse position	8.256	1	0.0041
Mpol population	247.05	1	2.20E-16

analyses. Median coverage depth for this primary filtered data set is 41 sequenced reads/locus, with among individual minimum and maximum sequencing depths of 8 and 72, respectively (Supporting Information Figure S3). The mean missing rate and heterozygosity per locus among all individuals are 5.55% and 12.6%, respectively and 12.6, respectively (Supporting Information Table S3).

Of the 9,658 SNPs, 98.5% (9514) were biallelic, 1.5% (142) were tri-allelic, and 0.0002% (2) were tetra-allelic. Excessive heterozygosity was observed in 156 loci (Supporting Information Figure S2), and 4,601 loci had minor allele frequencies <1.5% (~6 individuals) (Supporting Information Figure S2). These rare minor alleles are unlikely to be sequencing errors due to both strict read quality filtering during preprocessing and conservative SNP-calling parameters in GBSs. Heterozygosity among loci and individuals was similar for data sets that included or excluded loci that are rare, multi-allelic or display excess heterozygosity (observed heterozygosity, Supporting Information Table S3).

3.3 | Population structure

STRUCTURE 2.3.4 (Pritchard et al., 2000) was used to determine the population structure of *M. polymorpha* across its native and invaded ranges. Analysis of allele frequencies of 9,658 SNPs identified by GBS and application of Evanno's delta K method (Supporting Information Figure S3) reveals two clusters of *M. polymorpha* overall, with both clusters present in the native range and only one of the clusters present in the invaded range. Structure plots for $K = 3-6$ were also examined (Supporting Information Figure S3c). Native range populations are comprised of both clusters, with an observable geographical divide between northeasterly (Italy, France, northern Spain and Asia) populations that are predominantly Cluster 1 and southwesterly (Portugal, southern Spain, southern France) populations that are predominantly cluster 2 (Figure 2a). Individuals in the Middle East showed a heterogeneous mix of both clusters. Structure plots of native range individuals (Figure 2c, top) organized West (Portugal) to East (Italy) show an abrupt transition from individuals that are predominantly composed of Cluster 2 alleles, to individuals that are predominantly composed of Cluster 1 alleles. There is little variation in the proportion of Cluster 1 and Cluster 2 alleles among individuals within a population in the native range.

In contrast to the native range populations, all populations in the invaded range are composed predominantly of Cluster 2 alleles, with low levels of admixture from Cluster 1 (Figure 2b). Structure plots of invaded range individuals (Figure 2c, bottom) show that nearly all invaded range genotypes contain predominantly Cluster 2 alleles. The majority of SNP alleles in the northernmost deep GBS populations in Oregon and northern California (California 1 and 2) are from Cluster 2; however, Cluster 1 alleles were detected at a frequency of up to 40%. In contrast to this, SNP alleles in the deep GBS populations from Southern California and Florida are nearly all from Cluster 2. The deep GBS population sampled in Arizona shows that the majority of individuals are composed of predominantly Cluster 2 alleles; however, two individuals show a predominance of Cluster 1 alleles.

South American populations show a predominance of Cluster 2 alleles, similar to the North American populations.

Similar to findings with STRUCTURE, principal component analysis separates a cluster that includes both populations from the invaded range and from southwesterly native range populations (Portugal, southern Spain and southern France) from a cluster that includes northeasterly native range populations (Italy, northern France and northern Spain) along the PC1 axis. This axis explains 53.1% of variation (Figure 3). The PC2 axis separates invaded range populations from southwesterly native range populations and explains 14.5% of variation. Principal component analyses that excluded loci that are rare, multi-allelic or display excess heterozygosity showed highly similar clustering (Supporting Information Figure S5).

We calculated basic population genetic statistics for the total collection and for the native and invaded range genotypes in deep GBS populations (Figure 4, full set of G-statistics in Supporting Information Table S4). Total gene diversity (H_t) is higher in the native range ($H_t = 0.094$) as compared to the invaded range ($H_t = 0.057$). There are greater allele frequency differences between populations in the native range ($G_{st} = 0.563$) than between populations in the invaded range ($G_{st} = 0.358$). Lastly, there are similar levels of observed heterozygosity (H_o) between the native (0.016) and invaded (0.015) ranges. G-statistics for data subsets that exclude loci with rare minor alleles, excess heterozygosity, and multialleles are highly similar to those for the full data set (Supporting Information Table S4).

A greater proportion of the total genetic variance within a range is exhibited among populations in the native range as compared to among populations in the invaded range (Table 3). AMOVA results show that in the global population (combined native and invaded ranges), the highest proportion of variance is at the population level, followed by variation between individuals, and variance between ranges. This hierarchy is similar to that of the AMOVA of the native range separately, where the highest proportion of variance is at the population level (46.8%), followed by the individual within a population level. In contrast to this, the AMOVA of the invaded range shows that the highest proportion of variance is at the individual within population level, followed by the population level (31.4%). Data subsets that exclude loci with rare minor alleles, excess heterozygosity and multialleles do not substantially alter AMOVA results (Supporting Information Table S5).

Pairwise clustering indicates two major clusters of populations based on pairwise F_{st} . This divides the deep populations into the two STRUCTURE-defined groups (Figure 5a). All invaded range populations group within one cluster (defined as Cluster 2, similar to the STRUCTURE-designated populations). Also contained within this cluster are native populations from Portugal (Portugal 1–3), southern France (France 4) and southern Spain (Spain 4–5). Two adjacent populations in Florida (47.6 km between collection sites), Florida 1 and 2, also form a distinct cluster, separate from the other invaded range populations; however, these populations are composed of Cluster 2 alleles, similar to the remainder of the invaded range populations. The distribution of within- and between-cluster pairwise F_{st} values among deep GBS populations

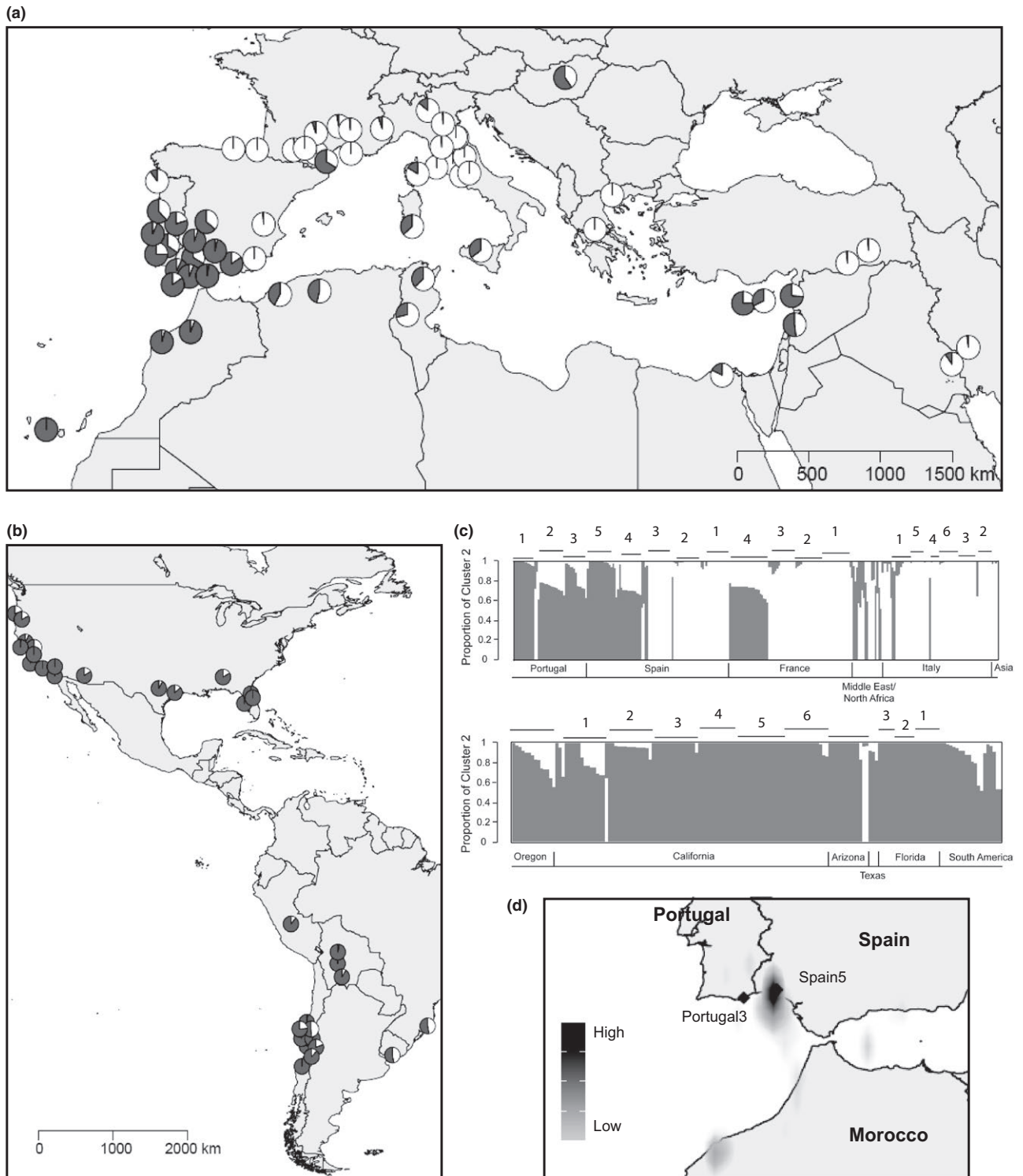


FIGURE 2 Population clustering by Structure analysis. Optimal number of clusters is based on $K = 2$, as determined by Evanno's delta K method (Evanno et al., 2005). (a) Proportion of Cluster 1 and Cluster 2 alleles in populations across the full native range. (b) Proportion of Cluster 1 and Cluster 2 alleles in populations across the full invaded range. Each pie chart represents the average of all individuals within one sampling location. White represents Cluster 1 alleles, and grey represents Cluster 2 alleles. Population locations in this map have been jittered to improve visualization; see Supporting Information Figure S5 for anunjittered version. (c) Comparison of the proportion of Cluster 1 and Cluster 2 alleles in all individuals within the full population by StructurePlot (Ramasamy, Ramasamy, Bindroo, & Naik, 2014). Top: native range individuals; bottom: invaded range individuals. Deep populations are indicated by the top brackets, and y-axis depicts the proportion of Cluster 2 alleles in both plots. (d) Results of population assignment tests, showing a heatmap of Scat2-assigned coordinates of invaded range *Medicago polymorpha* individuals. Genodive results mapped 96% of invaded range individuals back to the Portugal 3 population (labelled). Supporting Information Figure S8 shows the exact coordinates assigned to all invaded range individuals

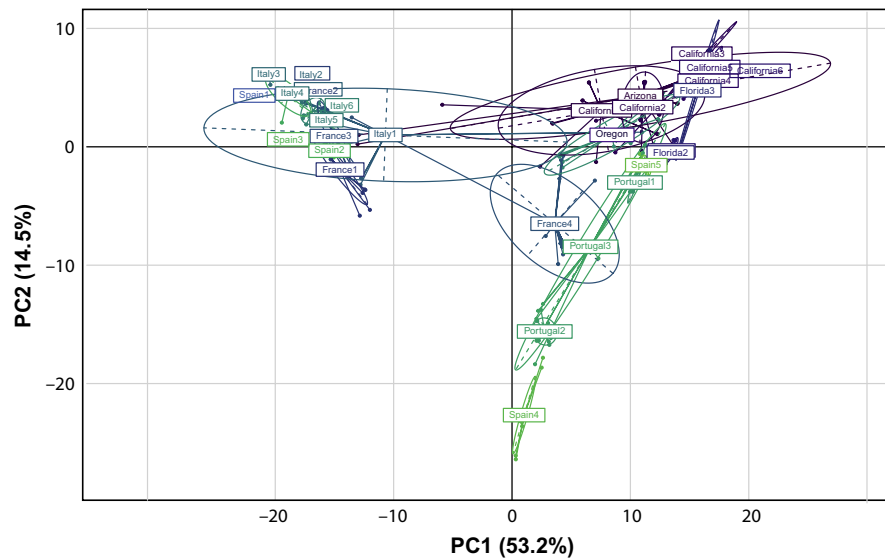


FIGURE 3 Principal component analysis (PCA) plot of deep GBS populations. Each point represents one individual, colours assigned by population, and ellipses cover 95% of individuals within a population. Population names are jittered for visibility

shows a distinct right-shifted peak of high between-cluster values (Figure 5b). This is congruent with other results that distinguish among these same *STRUCTURE*-defined groups. Supporting Information Figure S6 shows separate heatmaps for the native and invaded ranges, demonstrating that the native range separates into two significant clusters between the northeasterly populations and southwesterly populations.

Patterns of relatedness shown in the neighbornet (Figure 6) are consistent with the clustering of the pairwise F_{st} heatmap of the deep GBS population. The neighbornet is approximately divided in half, with the populations showing a predominance of Cluster 1 alleles (Italy, northern Spain, northern France and Asia) on one side of the neighbornet, and populations showing a predominance of Cluster 2 alleles (all invaded range populations, southern Spain, southern France and Portugal) on the other. Supporting Information Figure S7 shows a high-resolution neighbornet diagram with detailed labels for each tip.

Population assignment tests map the source of invaded *M. polymorpha* to a region encompassing Portugal and southern Spain. The two methods we used to assign individuals from the invaded range to their native population of origin showed broad agreement (Figure 2d) in assigning the origin of invaded range genotypes within southern Spain and Portugal, and northern Morocco. Genodive assigned 96% of invaded range individuals (130 out of 136) to a population located in Fuseta, Portugal (Portugal 3). *SCAT2* software assigned 135 out of 160 (84.4%) of the individuals in the invaded range to a region close to Portugal 3, along the southern coast of Spain, near Huelva (Spain 5). There were rare exceptions to these trends, seen in both the heatmap (Figure 2d) and Supporting Information Figure S8, showing the exact coordinates for each assigned individual. Genodive assigned with 2 out of 12 individuals in the Arizona population to the France 3 population and 4 out of 13 individuals in the California 1 population to the Italy 1 population. While individuals from California 2, 4 and 5 were all assigned to a small region near Spain 5, the remainder of the invaded range populations showed individuals that assigned to Morocco.

3.4 | Genome-wide association study

Acceptable Q-Q plots were obtained for GWAS models using the EMMA kinship model with untransformed flowering time data for (a) the full deep GBS population, (b) the native subset and (c) the invaded range subset (Supporting Information Figure S9). The full deep GBS collection and the native range subset returned the same 13 SNPs with significant associations with flowering time at a threshold of 0.05 FDR (Supporting Information Table S6). Mapping the GBS reads containing significant SNPs to the *Medicago truncatula*

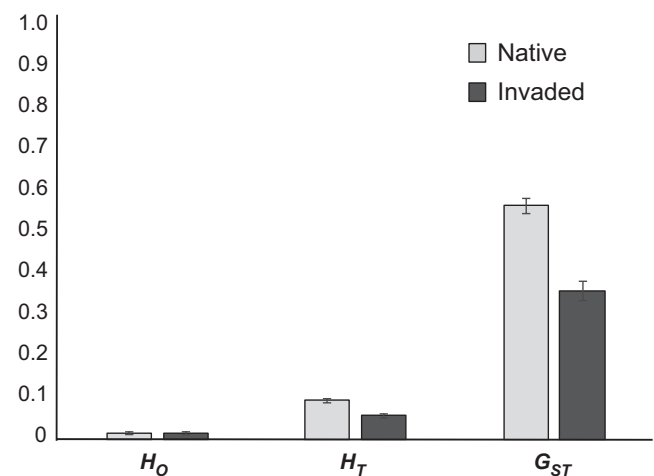


FIGURE 4 Patterns of genetic diversity among deep populations. Native: All individuals from the native range; invaded: all individuals from the invaded range (North America); H_O : observed heterozygosity; H_T : total gene diversity; G_{ST} : the correlation between gametes chosen randomly from within the same population relative to the range to which it belongs (Wright's F_{ST}). Error bars indicate the 97.5% confidence intervals, calculated by jackknifing over loci

TABLE 3 Partitioning of variance in the deep GBS populations. The variance of each population level for the combined native and invaded populations (overall), the native populations and the invaded populations, and the percent of the total variation for the combined native and invaded populations (overall), the native populations and the invaded populations. All terms are significant at $p < 0.05$

Population	Variance			Percent		
	Overall	Native	Invaded	Overall	Native	Invaded
Between Range	208.1	NA	NA	16.9	NA	NA
Between population within range	441.9	563.7	219.5	35.9	46.8	31.4
Between samples within population	428.6	482.3	333.6	34.8	40.1	47.8
Within samples	152.5	156.7	145.2	12.4	13.1	20.8
Total	1231.2	1202.8	698.3	100	100	100

genome identifies five hits falling within three distinct genes (Medtr3g096640.1 (DNA topoisomerase), Medtr6g035010.1 (beta-amyrin synthase) and Medtr6g008970.1 (paired amphipathic helix SIN3-like protein)); the remainder fall outside of genes (Supporting Information Table S7). All five SNPs are located within intronic regions of these genes and so are not predicted to impact amino acid sequence. There were no significant SNPs in invaded range individuals, and SNPs significant in the native range displayed low diversity in the invaded range (Supporting Information Table S3).

4 | DISCUSSION

Clinal variation in adaptive traits can be key to the successful spread of invasive species across large geographical regions (Bossdorf et al., 2005; Colautti & Lau, 2015; Prentis et al., 2008; Thompson, 1998). However, it is unclear whether latitudinal clines observed during species invasions originate from the direct translocation of clinal genetic variation from across the species' native latitudinal extent, versus a rapid establishment of a cline in the invaded range from genetic variation derived from a narrow source region. We observe genetically based latitudinal flowering time clines for the invasive plant *Medicago polymorpha* across its European native range and across its invaded range in North America. Yet population genetic structure based on a genome-wide set of 9,658 polymorphic SNPs indicates that invaded range populations spanning 80 degrees of latitude originate from founders with origins across only 11 degrees of latitude that runs through southern Portugal, Spain and northern Morocco. Furthermore, invaded range populations exhibit substantially lower genetic diversity than their native range counterparts. Our study adds to growing evidence that invasive species may adapt to local environmental conditions as they spread across large geographical ranges, despite relatively low genetic diversity (Colautti & Barrett, 2013; Samis et al., 2012). Lastly, we use an exploratory GWAS analysis to nominate several genes that could be associated with flowering time in the native, but not the invaded range.

4.1 | Latitudinal flowering cline despite a population bottleneck

Climatic factors vary continuously across latitude, and thus, clines in phenological traits including germination and flowering time are

predicted to play an important role in plant adaptation (e.g., Huxley, 1938; Endler 1977). Phenological traits that vary clinally have been observed in a wide range of broadly distributed plant taxa (Clevering, Brix, & Lukavská, 2001; Jonas & Geber, 1999; Li, Suzuki, & Hara, 1998; Olsson & Ågren, 2002; Stinchcombe et al., 2004; Van Dijk, Boudry, McComb, & Vernet, 1997; Van Dijk & Hautekèete, 2014; Winn & Gross, 1993), as well as several important crops (Erskine et al., 1994; Berger et al., 2004; Buckler et al., 2009; Jones et al., 2008; Varshney et al., 2017). Furthermore, several invasive species exhibit flowering time clines (e.g., *Eschscholzia californica*, California poppy, Leger & Rice, 2007; *Lythrum salicaria*, purple loosestrife, Montague et al., 2008; Colautti & Barrett, 2013; *Lactuca serriola*, wild lettuce, Alexander, 2013). What has been unclear in many invasion studies is whether the clines in the invaded range result from the rapid evolution of local adaptation or multiple introductions from different parts of the native range where clines would have formed over longer time periods (Colautti, Maron, & Barrett, 2009). By combining a common garden study of both native and invaded range plants coupled with reduced-representation genotyping-by-sequencing, we show that the flowering time cline in an invasive species' invaded range has formed despite a bottleneck following arrival from a narrow region of the native range.

Our study demonstrates that *M. polymorpha*, a globally widespread invasive weed, has spread across a wide geographical range and established a flowering cline in its invaded range, despite a strong reduction in genetic diversity. Our flowering cline results are consistent with earlier findings in which populations from Chile were found to show a latitudinal flowering cline (Del Pozo et al., 2002). Our results show individuals from low latitudes flower earlier than those from higher latitudes, in both the native and invaded range. Invaded range genotypes show a 60% reduction in total genetic diversity, as well as a 47% increase in allele fixation. Population genetic analyses show that *M. polymorpha* from its invaded range is not genetically distinct from *M. polymorpha* from its native range—instead there is a single population cluster that encompasses the invaded range and the southwest portion of the native range. Although we detect two clusters globally using STRUCTURE, these do not separate invaded and native ranges, but instead provide evidence of historic admixture. For many successful invasive species, multiple introductions from geographically distinct areas bolster genetic variation and increase the likelihood that admixture may account for successful range expansion (Ellstrand &

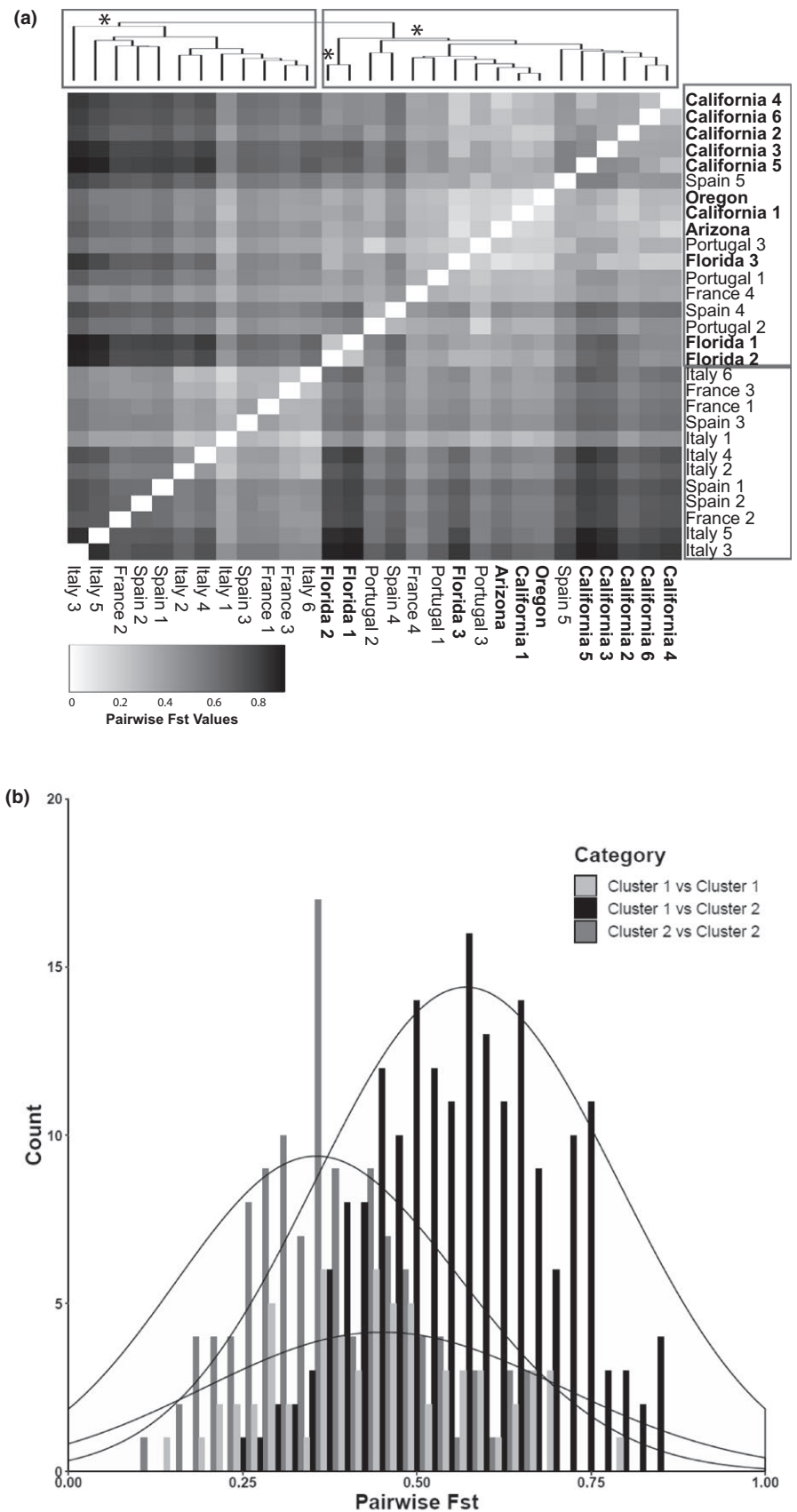


FIGURE 5 Pairwise F_{st} values between deep GBS populations. (a) Heatmap illustrating pairwise F_{st} values between all deep GBS populations. Pairwise F_{st} values are calculated by the Weir and Cockerham estimate (Weir & Cockerham, 1984). Invaded range populations are bolded. Asterisks indicate significant clusters of bootstrap values above 95. Boxes highlight populations that clustered together. (b) Histogram showing the distribution of pairwise F_{st} values among populations within- and between-clusters delineated in the heatmap. Supporting Information Figure S6 shows the pairwise F_{st} values between populations within the native and invaded ranges separately

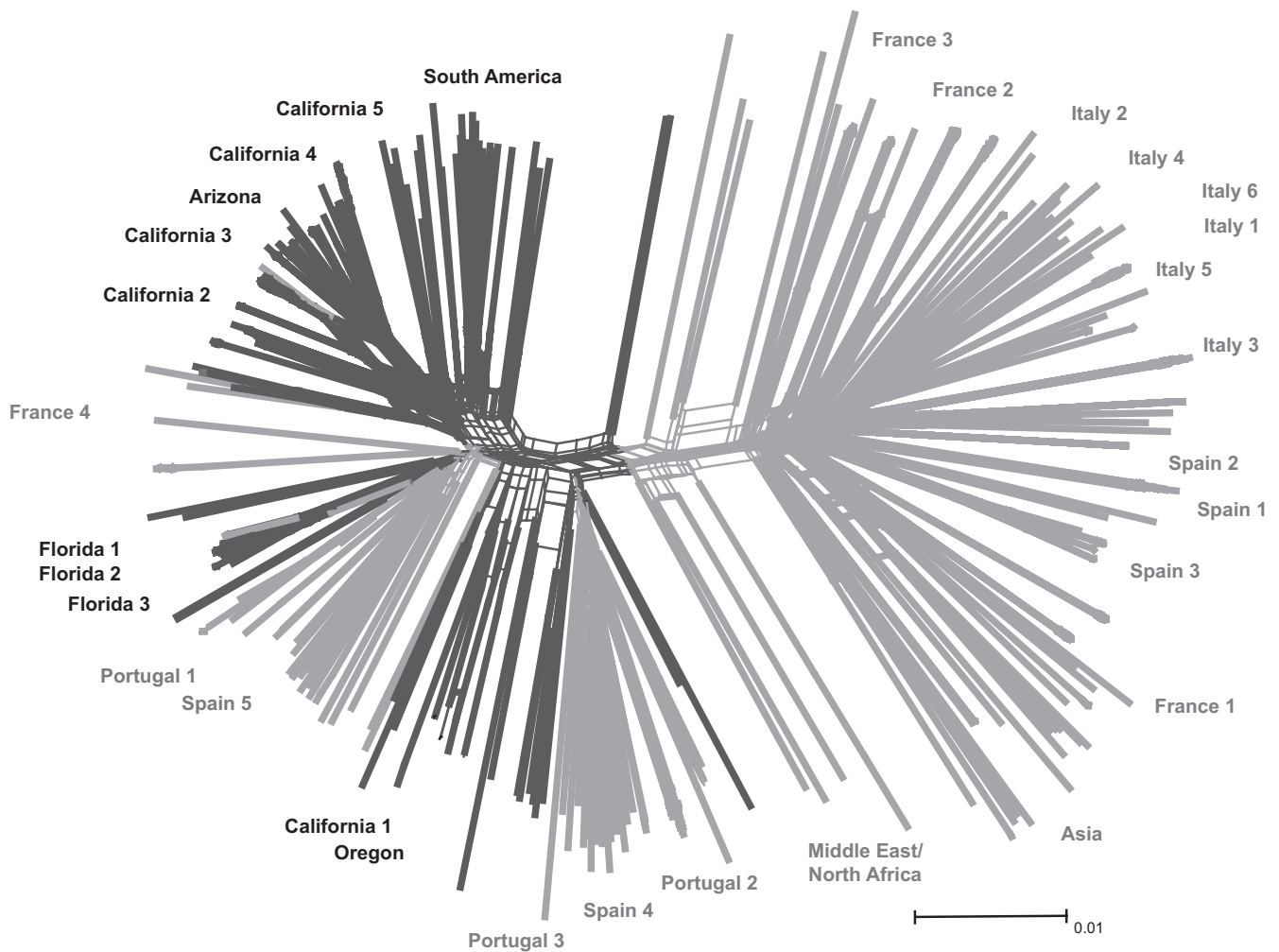


FIGURE 6 NeighborNet of all 446 individuals from the full population. Grey individuals are from the native range (Europe, Middle East/North Africa, Asia), and black individuals are from the invaded range (North America, South America, Australia). Clustering of deep populations including South America and Middle East/North Africa is labelled at the presence of four or more individuals of each population. Scale bar corresponds to the genetic distances between individuals. For greater resolution and tip identities, see Supporting Information Figure S7

Schierenbeck, 2000; Lee, 2002; Estoup et al., 2016). In contrast, our finding for *M. polymorpha* adds to a growing list of biological invasions that have spread beyond the founding location and may have adapted to conditions across a larger geographical range despite a bottleneck that has reduced genetic diversity (Hollingsworth and Bailey, 2000; Dlugosch & Parker, 2008; Richards et al., 2008; Loomis & Fishman, 2009; Richards, Schrey, & Pigliucci, 2012).

4.2 | Origin of invaded range *Medicago polymorpha*

Our findings suggest that a narrow region near the Strait of Gibraltar, including the Spanish port of Cadiz, is the region of origin of the invasive populations of *M. polymorpha* we examined. Two separate population assignment tests to determine the point(s) of origin for invaded range *M. polymorpha* assign North and South American individuals back to a narrow geographical range centred around the Strait of Gibraltar. The Genodive analysis assigns 95% of invaded range individuals to

the native range Portugal 3 population, located near Fuseta, Portugal, and the Scat2 analysis assigns 85% of invaded range individuals to the nearby native range Spain 5 population, near Huelva, Spain. Both sites are near ports where Spanish ships departed for the Americas during early European settlement of the Americas, making them likely sites of early introduction and colonization (Richardson et al., 2000). This is consistent with historical records linking colonizers from southern Spain as the vector of transport for this species—the earliest records of such translocation include still-viable *M. polymorpha* seeds embedded in adobe mud bricks from Spanish colonies in Northern Mexico from the 1700s (Spira & Wagner, 1983). The locations we sampled in Florida are near the first Spanish settlement in North America (St. Augustine) or in smaller coastal ports that would have been easily accessible by ships (Polatka and Tampa) (Tebeau, 1971). Our other populations east of the North American continental divide are also in areas that were Spanish possessions. Texas was controlled by Spain until 1821, and Spain briefly controlled southern Alabama from 1780 until 1813. Our

western North American populations are farther from early Spanish ports in the Americas, consistent with the further ecological spread of *M. polymorpha* in the more Mediterranean climates of western North America. Given corroboration between the two population assignment tests, the STRUCTURE analyses, and the pairwise F_{st} clustering showing overall similarity to southern Spain and Portugal populations, we identify the relatively narrow geographical region encompassing southern Portugal and Spain as the likely origin of the *Medicago polymorpha* invasion into the Americas.

In contrast to a climate-matching scenario with introductions from sources spanning the ancestral latitudinal range, our results suggest that *M. polymorpha* has undergone evolution in order to re-establish a latitudinal flowering cline across North America. Biological invasions are predicted to generally involve climate matching, where exotics thrive in climatic conditions similar to those in their native range such as from one Mediterranean region to another (Duncan, Bomford, Forsyth, & Conibear, 2001; Peterson, 2003; Thuiller, Lavorel, Araújo, Sykes, & Prentice, 2005; Bomford, Barry, & Lawrence, 2010; Petitpierre et al., 2012). Climate matching is sufficiently important that it is a central criterion in laws controlling introduction of ornamental species in many countries and regions such as Australia, New Zealand and several Pacific Islands (Pheloung, Williams, & Halloy, 1999; Daehler, Denslow, Ansari, & Kuo, 2004; Rogg, Buddenhagen, & Causton, 2003–2005). Nevertheless, for many invasive species, climate matching does not accurately predict the regions where invasive species can thrive (Alexander, 2013; Broennimann et al., 2007; Gallagher, Beaumont, Hughes, & Leishman, 2010; Mandle et al., 2010). Climate matching may not occur for many reasons, from artefacts or biases in the environmental niche models often used to assess climate matching (Araujo & Peterson, 2012; Mandle et al., 2010), from the lack of a particular climatic space in the native range (Mandle et al., 2010) or from evolution of different niche breadths. The introduction of *M. polymorpha* along a wide latitudinal range spanning Mediterranean-like climates from Chile to the western United States appears to have occurred from a very narrow area of origin within the native range flowering time cline, rather than due to transport of the cline intact from across the extent of its latitudinal distribution in the native range. This supports a scenario under which rapid evolution may have occurred during cline establishment, and prompts caution when using climate matching to assess invasion risk in rapidly evolving annual plants.

4.3 | Candidate genes underlying flowering time variation

Flowering time is a model trait for understanding the genetics of clinal adaptation (Mouradov, Cremer, & Coupland, 2002; Pineiro & Coupland, 1998; Weller & Ortega, 2015), and the evolution of clines in flowering time has been broadly implicated in the invasion biology of annual plants. Several genes have been implicated as playing roles in the formation of latitudinal as well as longitudinal flowering clines in *Arabidopsis thaliana* in both its native (Balasubramanian et al., 2006;

Caicedo, Stinchcombe, Olsen, Schmitt, & Purugganan, 2004; Samis, Heath, & Stinchcombe, 2008; Stinchcombe et al., 2004) and invaded range (Samis et al., 2012). In the model legume, *Medicago truncatula*, a dozen genes from several pathways involved in floral development are associated with clinal variation in flowering time (Burgarella et al., 2016). We thus sought to identify whether GBS data in the close relative, *M. polymorpha*, showed genetic associations with flowering time, although this provided a low-powered test with only ~10K markers. Assuming *M. polymorpha* has a ~500 Mbp genome size close to that of *M. truncatula* (Young et al., 2011), and genome-wide LD of ~10Kbp similar to *M. truncatula* (Friesen et al., 2014), then our markers only capture ~100Mbp which is about 20% of the genome. Given that *M. polymorpha* typically has larger population sizes and reduced population structure compared to *M. truncatula* (Badri, Cheikh, Mahjoub, & Abdely, 2016), we expect that LD is substantially lower than this and thus our power may be further reduced. Therefore, we proceed cautiously in our interpretation of GWAS results.

Despite these caveats, we observed 10 SNPs that assort with clinal variation in flowering time in native range populations, though not in invaded range populations. Of these, five candidate SNPs fall within three genes, including one homologous to the redundant *A. thaliana* SIN3-like 1 gene, two genes annotated as DNA topoisomerases, and two genes annotated as beta-amyrin synthases (Supporting Information Table S6). While this finding is consistent with a scenario in which populations in the native and invaded range adapt to climatic differences via distinct genetic mechanisms, given the limited number of markers in our study and the fact that we examined fewer individuals in the invaded range as compared to the native range, we consider this a preliminary finding. Alternatively, flowering time could be impacted by heritable epigenetic changes, especially given the genetic bottleneck and wide phenotypic range observed in invaded range lineages. The density of markers that could be provided by whole-genome resequencing would allow us to more robustly investigate these possibilities in future studies.

5 | CONCLUSION

Flowering time is a key life history trait that can have a major impact on fitness and is thus likely a target of strong selection during invasion and spread across large geographical areas. Data from a common garden experiment and population genomic approaches reveal multiple lines of evidence that *Medicago polymorpha* invaded North America from source populations within a narrow region in southern Spain and Portugal. The species then established a latitudinal flowering cline while spreading across a wide geographical region in America, despite a reduction in genetic variation in the invaded range. Therefore, our data support a scenario in which rapid evolution in flowering time has occurred despite a genetic bottleneck following introduction. Our overall findings add to the growing knowledge of the different strategies used by invasive species to establish and rapidly spread across wide geographical regions.

ACKNOWLEDGEMENTS

We thank D. Cook and N. Carrasquilla-Garcia for assistance with DNA extraction and acknowledge the Cornell Genomic Diversity Facility for technical support with genotyping-by-sequencing. C. Sackos, D. Ross, C. Zielinski and G. Davis assisted with plant propagation. Y. Porter provided assistance with field collections. EvW acknowledges support from the University of Vermont State Agriculture Experimental Station and the USDA Hatch programme. This work was funded by NSF DEB-1355216 to S.S. Porter and NSF DEB-1354878 to M.L. Friesen.

6 | DATA ACCESSIBILITY

GBS sequencing data are available under NCBI BioProject PRJNA492754. SNP data set and flowering time data sets are available at Dryad (<https://doi.org/doi:10.5061/dryad.79928sj>).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

E.E.H. wrote and organized the article; J.F.H., Z.C.L., A.G., EvW., M.L.F. and S.S.P. contributed to the writing of the article; E.E.H., J.F.H. and Z.C.L. prepared the tables and figures; E.E.H. analysed the population genetics data, J.F.H. analysed the G.B.S. data; Z.C.L. analysed the phenotypic data; and A.G. analysed the G.W.A.S. data; Z.C.L. and S.S.P. performed the common garden experiments; EvW., M.L.F. and S.S.P. designed the experiments; Z.C.L., EvW., M.L.F. and S.S.P. collected biological material; and EvW., M.L.F. and S.S.P. reviewed drafts of the article.

ORCID

Emily E. Helliwell  <http://orcid.org/0000-0001-6340-7260>

REFERENCES

- Alexander, J. M. (2013). Evolution under changing climates: Climatic niche stasis despite rapid evolution in a non-native plant. *Proceedings of the Royal Society B: Biological Sciences*, 280. <https://doi.org/10.1098/rspb.2013.1446>
- Araujo, M. B., & Peterson, T. A. (2012). Uses and misuses of bioclimatic envelope modeling. *Ecology*, 93, 1527–1539. <https://doi.org/10.1890/11-1930.1>
- Arft, A. M., Walker, M. D., Gurevitch, J., Alatalo, J. M., Bret-Harte, M. S., Dale, M., ... Wookey, P. A. (1999). Responses of tundra plants to experimental warming: Meta-analysis of the international tundra experiment. *Ecological Monographs*, 69, 491–511.
- Badri, M., Cheikh, N. B., Mahjoub, A., & Abdelly, C. (2016). Morpho-phenological diversity among natural populations of *Medicago polymorpha* of different Tunisian ecological areas. *African Journal of Biotechnology*, 15(25), 1330–1338.
- Balasubramanian, S., Sureshkumar, S., Agrawal, M., Michael, T. P., Wessinger, C., Maloof, J. N., ... Weigel, D. (2006). The PHYTOCHROME C photoreceptor gene mediates natural variation in flowering and growth responses of *Arabidopsis thaliana*. *Nature Genetics*, 38, 711–715. <https://doi.org/10.1038/ng1818>
- Barker, B. S., Andonian, K., Swope, S. M., Luster, D. G., & Dlugosch, K. M. (2017). Population genomic analyses reveal a history of range expansion and trait evolution across the native and invaded range of yellow starthistle (*Centaurea solstitialis*). *Molecular Ecology*, 26, 1131–1147. <https://doi.org/10.1111/mec.13998>
- Berger, J. D., Turner, N. C., Siddique, K. H. M., Knights, E. J., Brinsmead, R. B., Mock, I., ... Khan, T. N. (2004). Genotype by environment studies across Australia reveal the importance of phenology for chickpea (*Cicer arietinum* L.) improvement. *Australian Journal of Agricultural Research*, 55, 1071–1084. <https://doi.org/10.1071/AR04104>
- Bomford, M., Barry, S. C., & Lawrence, E. (2010). Predicting establishment success for introduced freshwater fishes: A role for climate matching. *Biological Invasions*, 12, 2559–2571. <https://doi.org/10.1007/s10530-009-9665-3>
- Bossdorf, O., Auge, H., Lafuma, L., Rogers, W. E., Siemann, E., & Prati, D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, 144, 1–11. <https://doi.org/10.1007/s00442-005-0070-z>
- Bouché, F., Woods, D. P., & Amasino, R. M. (2017). Winter memory throughout the plant kingdom: Different paths to flowering. *Plant Physiology*, 173, 27–35. <https://doi.org/10.1104/pp.16.01322>
- Broennimann, O., Treier, U. A., Müller-Schärer, H., Thuiller, W., Peterson, A. T., & Guisan, A. (2007). Evidence of climatic niche shift during biological invasion. *Ecology Letters*, 10, 701–709. <https://doi.org/10.1111/j.1461-0248.2007.01060.x>
- Bryant, D. (2003). Neighbor-net: An agglomerative method for the construction of phylogenetic networks. *Molecular Biology and Evolution*, 21, 255–265. <https://doi.org/10.1093/molbev/msh018>
- Buckler, E. S., Holland, J. B., Bradbury, P. J., Acharya, C. B., Brown, P. J., Browne, C., ... Goodman, M. M. (2009). The genetic architecture of maize flowering time. *Science*, 325(5941), 714–718.
- Burgarella, C., Chantret, N., Gay, L., Prosperi, J.-M., Bonhomme, M., Tiffin, P., ... Ronfort, J. (2016). Adaptation to climate through flowering phenology: A case study in *Medicago truncatula*. *Molecular Ecology*, 25, 3397–3415. <https://doi.org/10.1111/mec.13683>
- Caicedo, A. L., Stinchcombe, J. R., Olsen, K. M., Schmitt, J., & Purugganan, M. D. (2004). Epistatic interaction between *Arabidopsis* FRI and FLC flowering time genes generates a latitudinal cline in a life history trait. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 15670–15675. <https://doi.org/10.1073/pnas.0406232101>
- Clevering, O. A., Brix, H., & Lukavská, J. (2001). Geographic variation in growth responses in *Phragmites australis*. *Aquatic Botany*, 69, 89–108. [https://doi.org/10.1016/S0304-3770\(01\)00132-2](https://doi.org/10.1016/S0304-3770(01)00132-2)
- Colautti, R. I., & Barrett, S. C. H. (2010). Natural selection and genetic constraints on flowering phenology in an invasive plant. *International Journal of Plant Sciences*, 171, 960–971. <https://doi.org/10.1086/656444>
- Colautti, R. I., & Barrett, S. C. (2013). Rapid adaptation to climate facilitates range expansion of an invasive plant. *Science*, 342, 364–366. <https://doi.org/10.1126/science.1242121>
- Colautti, R. I., & Lau, J. A. (2015). Contemporary evolution during invasion: Evidence for differentiation, natural selection, and local adaptation. *Molecular Ecology*, 24, 1999–2017. <https://doi.org/10.1111/mec.13162>
- Colautti, R. I., Maron, J. L., & Barrett, S. C. H. (2009). Common garden comparisons of native and introduced plant populations: Latitudinal clines can obscure evolutionary inferences. *Evolutionary Applications*, 2, 187–199. <https://doi.org/10.1111/j.1752-4571.2008.00053.x>
- Craig, D. W., Pearson, J. V., Szelinger, S., Sekar, A., Redman, M., Corneveaux, J. J., ... Huentelman, M. J. (2008). Identification of

- genetic variants using bar-coded multiplexed sequencing. *Nature Methods*, 5, 887–893. <https://doi.org/10.1038/nmeth.1251>
- Daehler, C. C., Denslow, J. S., Ansari, S., & Kuo, H.-C. (2004). A risk-assessment system for screening out invasive pest plants from Hawaii and other Pacific Islands. *Conservation Biology*, 18, 360–368.
- Davis, M. B., Shaw, R. G., & Etterson, J. R. (2005). Evolutionary responses to changing climate. *Ecology*, 86, 1704–1714. <https://doi.org/10.1890/03-0788>
- Del Pozo, A., Ovalle, C., Aronson, J., & Avendaño, J. (2002). Ecotypic differentiation in *Medicago polymorpha* L. along an environmental gradient in central Chile. I. Phenology, biomass production and reproductive patterns. *Plant Ecology*, 159, 119–130. <https://doi.org/10.1023/A:1015506914038>
- Drugosch, K. M., Anderson, S. R., Braasch, J., Cang, F. A., & Gillette, H. D. (2015). The devil is in the details: Genetic variation in introduced populations and its contributions to invasion. *Molecular Ecology*, 24, 2095–2111. <https://doi.org/10.1111/mec.13183>
- Drugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: Genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, 17, 431–449. <https://doi.org/10.1111/j.1365-294X.2007.03538.x>
- Duncan, R. P., Bomford, M., Forsyth, D. M., & Conibear, L. (2001). High predictability in introduction outcomes and the geographical range size of introduced Australian birds: A role for climate. *Journal of Animal Ecology*, 70, 621–632. <https://doi.org/10.1046/j.1365-2656.2001.00517.x>
- Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A web-site and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4, 359–361. <https://doi.org/10.1007/s12686-011-9548-7>
- Ehrman, T., & Cocks, P. S. (1996). Reproductive patterns in annual legume species on an aridity gradient. *Vegetatio*, 122, 47–59. <https://doi.org/10.1007/BF00052815>
- Ellstrand, N. C., & Schierenbeck, K. A. (2000). Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences of the United States of America*, 97, 7043–7050. <https://doi.org/10.1073/pnas.97.13.7043>
- Endler, J. A. (1977). *Geographic variation, speciation, and clines* (No. 10). Princeton, NJ: Princeton University Press.
- Erskine, W., Hussain, A., Tahir, M., Bahksh, A., Ellis, R. H., Summerfield, R. J., & Roberts, E. H. (1994). Field evaluation of a model of photothermal flowering responses in a world lentil collection. *Theoretical and Applied Genetics*, 88–88, 3–4.
- Estoup, A., Ravigné, V., Hufbauer, R., Vitalis, R., Gautier, M., & Facon, B. (2016). Is there a genetic paradox of biological invasion? *Annual Review of Ecology, Evolution, and Systematics*, 47, 51–72. <https://doi.org/10.1146/annurev-ecolsys-121415-032116>
- Etterson, J. R., Toczydlowski, R. H., Winkler, K. J., Kirschbaum, J. A., & McAulay, T. S. (2016). Solidago altissima differs with respect to ploidy frequency and clinal variation across the prairie-forest biome border in Minnesota. *American Journal of Botany*, 103, 22–32. <https://doi.org/10.3732/ajb.1500146>
- Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, 14, 2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Fox, J., & Weisberg, S. (2011). *An R companion to applied regression*, 2nd edn. Thousand Oaks, CA: Sage.
- Friesen, M. L., von Wettberg, E. J., Badri, M., Moriuchi, K. S., Barhoumi, F., Chang, P. L., ... Arraouadi, S. (2014). The ecological genomic basis of salinity adaptation in Tunisian *Medicago truncatula*. *BMC Genomics*, 15, 1160. <https://doi.org/10.1186/1471-2164-15-1160>
- Galibert, F., Finan, T. M., Long, S. R., Pühler, A., Abola, P., Ampe, F., ... Batut, J. (2001). The composite genome of the legume symbiont *Sinorhizobium meliloti*. *Science*, 293, 668–672. <https://doi.org/10.1126/science.1060966>
- Gallagher, R. V., Beaumont, L. J., Hughes, L., & Leishman, M. R. (2010). Evidence for climatic niche and biome shifts between native and novel ranges in plant species introduced to Australia: Climate niches and exotic species. *Journal of Ecology*, 98, 790–799. <https://doi.org/10.1111/j.1365-2745.2010.01677.x>
- Gilchrist, G. W., Huey, R. B., & Serra, L. (2001). Rapid evolution of wing size clines in *Drosophila subobscura*. *Genetica*, 112–113, 273–286.
- Glaubitz, J. C., Casstevens, T. M., Lu, F., Harriman, J., Elshire, R. J., Sun, Q., & Buckler, E. S. (2014). TASSEL-GBS: A high capacity genotyping by sequencing analysis pipeline. *PLoS ONE*, 9(2), e90346. <https://doi.org/10.1371/journal.pone.0090346>
- Goudet, J. (2005). Hierfstat, a package for R to compute and test hierarchical F-statistics. *Molecular Ecology Resources*, 5, 184–186.
- Hapke, A., & Thiele, D. (2016). GlibPSS: A toolkit for fast and accurate analyses of genotyping-by-sequencing data without a reference genome. *Molecular Ecology Resources*, 16, 979–990. <https://doi.org/10.1111/1755-0998.12510>
- Hollingsworth, M. L., & Bailey, J. P. (2000). Evidence for massive clonal growth in the invasive weed *Fallopia japonica* (Japanese knotweed). *Botanical Journal of the Linnean Society*, 133, 463–472.
- Hudson, D. H. (1998). SplitsTree: A program for analyzing and visualizing evolutionary data. *Bioinformatics*, 14, 68–73. <https://doi.org/10.1093/bioinformatics/14.1.68>
- Huey, R. B., Gilchrist, G. W., Carlson, M. L., Berrigan, D., & Serra, L. (2000). Rapid evolution of a geographic cline in size in an introduced fly. *Science*, 287, 308–309. <https://doi.org/10.1126/science.287.5451.308>
- Huxley, J. S. (1938). Species formation and geographical isolation. *Proceedings Linnean Society London*, 150(4), 253–264. <https://doi.org/10.1111/j.1095-8312.1938.tb00182g.x>
- Jombart, T., & Ahmed, I. (2011). adegenet 1.3-1: New tools for the analysis of genome-wide SNP data. *Bioinformatics*, 27, 3070–3071. <https://doi.org/10.1093/bioinformatics/btr521> <https://doi.org/10.1093/bioinformatics/btr521>
- Jonas, C. S., & Geber, M. A. (1999). Variation among populations of *Clarkia unguiculata* (Onagraceae) along altitudinal and latitudinal gradients. *American Journal of Botany*, 86, 333–343. <https://doi.org/10.2307/2656755>
- Jones, H., Leigh, F. J., Mackay, I., Bower, M. A., Smith, L. M. J., Charles, M. P., ... Powell, W. (2008). Population-based resequencing reveals that the flowering time adaptation of cultivated barley originated east of the fertile crescent. *Molecular Biology and Evolution*, 25, 2211–2219. <https://doi.org/10.1093/molbev/msn167>
- Jung, C., & Müller, A. E. (2009). Flowering time control and applications in plant breeding. *Trends in Plant Science*, 14, 563–573. <https://doi.org/10.1016/j.tplants.2009.07.005>
- Kamvar, Z. N., Tabima, J. F., & Grünwald, N. J. (2014). Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, 2, e281. <https://doi.org/10.7717/peerj.281>
- Kazan, K., & Lyons, R. (2016). The link between flowering time and stress tolerance. *Journal of Experimental Botany*, 67, 47–60. <https://doi.org/10.1093/jxb/erv441>
- Kollmann, J., & Bañuelos, M. J. (2004). Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (Balsaminaceae). *Diversity and Distributions*, 10, 377–385. <https://doi.org/10.1111/j.1366-9516.2004.00126.x>
- Lee, C. E. (2002). Evolutionary genetics of invasive species. *Trends in ecology & evolution*, 17(8), 386–391.
- Leger, E. A., & Rice, K. J. (2007). Assessing the speed and predictability of local adaptation in invasive California poppies (*Eschscholzia californica*). *Journal of Evolutionary Biology*, 20, 1090–1103. <https://doi.org/10.1111/j.1420-9101.2006.01292.x>

- Lesins, K. A., & Lesins, I. (1979). *Genus Medicago* (Leguminosae): A taxogenetic study. Dr. W Junk, The Hague, The Netherlands. <https://doi.org/10.1007/978-94-009-9634-2>
- Li, B., Suzuki, J.-I., & Hara, T. (1998). Latitudinal variation in plant size and relative growth rate in *Arabidopsis thaliana*. *Oecologia*, 115, 293–301. <https://doi.org/10.1007/s004420050519>
- Lipka, A. E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P. J., ... Zhang, Z. (2012). GAPIT: Genome association and prediction integrated tool. *Bioinformatics*, 28, 2397–2399. <https://doi.org/10.1093/bioinformatics/bts444>
- Loomis, E. S., & Fishman, L. (2009). A continent-wide clone: Population genetic variation of the invasive plant *Hieracium aurantiacum* (Orange Hawkweed; Asteraceae) in North America. *International Journal of Plant Sciences*, 170, 759–765. <https://doi.org/10.1086/599241>
- Mandle, L., Warren, D. L., Hoffmann, M. H., Peterson, A. T., Schmitt, J., & von Wettberg, E. J. (2010). Conclusions about niche expansion in introduced *Impatiens walleriana* populations depend on method of analysis. *PLoS ONE*, 5, e15297. <https://doi.org/10.1371/journal.pone.0015297>
- Maron, J. L., Elmhendorf, S. C., & Vilà, M. (2007). Contrasting plant physiological adaptation to climate in the native and introduced range of *Hypericum perforatum*. *Evolution*, 61, 1912–1924. <https://doi.org/10.1111/j.1558-5646.2007.00153.x>
- Meirmans, P. G., & Van Tienderen, P. H. (2004). Genotype and Genodive: Two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, 4, 792–794. <https://doi.org/10.1111/j.1471-8286.2004.00770.x>
- Mitchell-Olds, T., & Schmitt, J. (2006). Genetic mechanisms and evolutionary significance of natural variation in *Arabidopsis*. *Nature*, 441, 947–952. <https://doi.org/10.1038/nature04878>
- Montague, J. L., Barrett, S. C. H., & Eckert, C. G. (2008). Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythrum salicaria*, Lythraceae): Evolution during biological invasion. *Journal of Evolutionary Biology*, 21, 234–245. <https://doi.org/10.1111/j.1420-9101.2007.01456.x>
- Mouradov, A., Cremer, F., & Coupland, G. (2002). Control of flowering time: Interacting pathways as a basis for diversity. *The Plant Cell*, 14, S111–S130. <https://doi.org/10.1105/tpc.001362>
- Olsson, K., & Ågren, J. (2002). Latitudinal population differentiation in phenology, life history and flower morphology in the perennial herb *Lythrum salicaria*. *Journal of Evolutionary Biology*, 15, 983–996. <https://doi.org/10.1046/j.1420-9101.2002.00457.x>
- Pairon, M., Petitpierre, B., Campbell, M., Guisan, A., Broennimann, O., Baret, P. V., ... Besnard, G. (2010). Multiple introductions boosted genetic diversity in the invasive range of black cherry (*Prunus serotina*; Rosaceae). *Annals of Botany*, 105, 881–890. <https://doi.org/10.1093/aob/mcq065>
- Patalano, S., Vlasova, A., Wyatt, C., Ewels, P., Camara, F., Ferreira, P. G., ... Sumner, S. (2015). Molecular signatures of plastic phenotypes in two eusocial insect species with simple societies. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 13970–13975. <https://doi.org/10.1073/pnas.1515937112>
- Peterson, A. T. (2003). Predicting the geography of species' invasions via ecological niche modeling. *The Quarterly Review of Biology*, 78, 419–433. <https://doi.org/10.1086/378926>
- Pinheiro, J. C., & Bates, D. (2009). *Mixed-Effects Models in S and SPLUS*. Springer: New York.
- Petitpierre, B., Kueffer, C., Broennimann, O., Randin, C., Daehler, C., & Guisan, A. (2012). Climatic niche shifts are rare among terrestrial plant invaders. *Science*, 335(6074), 1344–1348.
- Pheloung, P. C., Williams, P. A., & Halloy, S. R. (1999). A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. *Journal of Environmental Management*, 57, 239–251. <https://doi.org/10.1006/jema.1999.0297>
- Pineiro, M., & Coupland, G. (1998). The control of flowering time and floral identity in *Arabidopsis*. *Plant Physiology*, 117, 1–8. <https://doi.org/10.1104/pp.117.1.1>
- Porter, S. S., Stanton, M. L., & Rice, K. J. (2011). Mutualism and adaptive divergence: Co-invasion of a heterogeneous grassland by an exotic legume-rhizobium symbiosis. *PLoS ONE*, 6, e27935. <https://doi.org/10.1371/journal.pone.0027935>
- Prentis, P. J., Wilson, J. R. U., Dormontt, E. E., Richardson, D. M., & Lowe, A. J. (2008). Adaptive evolution in invasive species. *Trends in Plant Science*, 13, 288–294. <https://doi.org/10.1016/j.tplants.2008.03.004>
- Price, J., Harrison, M. C., Hammond, R. L., Adams, S., Gutierrez-Marcos, J. F., & Mallon, E. B. (2018). Alternative splicing associated with phenotypic plasticity in the bumble bee *Bombus terrestris*. *Molecular Ecology*, 27, 1036–1043. <https://doi.org/10.1111/mec.14495>
- Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.
- R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2016. <https://www.r-project.org/>
- Ramasamy, R. K., Ramasamy, S., Bindroo, B. B., & Naik, V. G. (2014). STRUCTURE PLOT: A program for drawing elegant STRUCTURE bar plots in user friendly interface. *SpringerPlus*, 3, 431. <https://doi.org/10.1186/2193-1801-3-431>
- Reeve, W., Chain, P., O'Hara, G., Ardley, J., Nandesena, K., Bräu, L., ... Lapidus, A. (2010). Complete genome sequence of the *Medicago* microsymbiont *Ensifer* (*Sinorhizobium*) *medicae* strain WSM419. *Standards in Genomic Sciences*, 2, 77. <https://doi.org/10.4056/sigs.43526>
- Richards, C. L., Schrey, A. W., & Pigliucci, M. (2012). Invasion of diverse habitats by few Japanese knotweed genotypes is correlated with epigenetic differentiation. *Ecology Letters*, 15, 1016–1025. <https://doi.org/10.1111/j.1461-0248.2012.01824.x>
- Richards, C. L., Walls, R. L., Bailey, J. P., Parameswaran, R., George, T., & Pigliucci, M. (2008). Plasticity in salt tolerance traits allows for invasion of novel habitat by Japanese knotweed s. l. (*Fallopia japonica* and *F.x bohemica*, Polygonaceae). *American Journal of Botany*, 95, 931–942. <https://doi.org/10.3732/ajb.2007364>
- Richardson, D. M., Pyšek, P., Rejmánek, M., Barbour, M. G., Panetta, F. D., & West, C. J. (2000). Naturalization and invasion of alien plants: Concepts and definitions. *Diversity and Distributions*, 6, 93–107. <https://doi.org/10.1046/j.1472-4642.2000.00083.x>
- Ridley, C. E., & Ellstrand, N. C. (2010). Rapid evolution of morphology and adaptive life history in the invasive California wild radish (*Raphanus sativus*) and the implications for management. *Evolutionary Applications*, 3, 64–76. <https://doi.org/10.1111/j.1752-4571.2009.00099.x>
- Rogg, H., Buddenhagen, C., & Causton, C. (2003–2005). *Experiences and limitations with pest risk analysis in the Galapagos Islands* (pp. 117–126). Baunschweig, Germany: FAO.
- Samis, K. E., Heath, K. D., & Stinchcombe, J. R. (2008). Discordant longitudinal clines in flowering time and Phytochrome C in *Arabidopsis thaliana*. *Evolution*, 62, 2971–2983. <https://doi.org/10.1111/j.1558-5646.2008.00484.x>
- Samis, K. E., Murren, C. J., Bossdorf, O., Donohue, K., Fenster, C. B., Malmberg, R. L., ... Stinchcombe, J. R. (2012). Longitudinal trends in climate drive flowering time clines in North American *Arabidopsis thaliana*: Longitudinal flowering time clines in North American *Arabidopsis*. *Ecology and Evolution*, 2, 1162–1180. <https://doi.org/10.1002/ece3.262>
- Singh, P., Börger, C., More, H., & Sturmbauer, C. (2017). The role of alternative splicing and differential gene expression in cichlid adaptive radiation. *Genome Biology and Evolution*, 9, 2764–2781. <https://doi.org/10.1093/gbe/evx204>
- Skelly, D. K., Joseph, L. N., Possingham, H. P., Freidenburg, L. K., Farrugia, T. J., Kinnison, M. T., & Hendry, A. P. (2007). Evolutionary responses to climate change. *Conservation Biology*, 21, 1353–1355. <https://doi.org/10.1111/j.1523-1739.2007.00764.x>
- Song, Y. H., Shim, J. S., Kinmonth-Schultz, H. A., & Imaizumi, T. (2015). Photoperiodic flowering: Time measurement mechanisms in leaves.

- Annual Review of Plant Biology*, 66, 441–464. <https://doi.org/10.1146/annurev-arplant-043014-115555>
- Spicer, R. A., & Chapman, J. L. (1990). Climate change and the evolution of high-latitude terrestrial vegetation and floras. *Trends in Ecology & Evolution*, 5, 279–284. [https://doi.org/10.1016/0169-5347\(90\)90081-N](https://doi.org/10.1016/0169-5347(90)90081-N)
- Spira, T. P., & Wagner, L. K. (1983). Viability of seeds up to 211 years old extracted from adobe brick buildings of California and Northern Mexico. *American Journal of Botany*, 70, 303–307. <https://doi.org/10.1002/j.1537-2197.1983.tb07871.x>
- Stinchcombe, J. R., Weinig, C., Ungerer, M., Olsen, K. M., Mays, C., Halldorsdottir, S. S., ... Schmitt, J. (2004). A latitudinal cline in flowering time in *Arabidopsis thaliana* modulated by the flowering time gene FRIGIDA. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 4712–4717. <https://doi.org/10.1073/pnas.0306401101>
- Suzuki, R., & Shimodaira, H. (2006). Pvcust: An R package for assessing the uncertainty in hierarchical clustering. *Bioinformatics*, 22, 1540–1542. <https://doi.org/10.1093/bioinformatics/btl117>
- Tang, H., Krishnakumar, V., Bidwell, S., Rosen, B., Chan, A., Zhou, S., ... Gundlach, H. (2014). An improved genome release (version Mt4. 0) for the model legume *Medicago truncatula*. *BMC Genomics*, 15, 312. <https://doi.org/10.1186/1471-2164-15-312>
- Tebeau, C. W. (1971). *A history of Florida*. Coral Gables, FL: University of Miami Press.
- Therneau, T. (2015). *A package for survival analysis in S. version 2.38*. Retrieved from <https://CRAN.R-project.org/package=survival>.
- Thompson, J. N. (1998). Rapid evolution as an ecological process. *Trends in Ecology & Evolution*, 13, 329–332. [https://doi.org/10.1016/S0169-5347\(98\)01378-0](https://doi.org/10.1016/S0169-5347(98)01378-0)
- Thuiller, W., Lavorel, S., Araújo, M. B., Sykes, M. T., & Prentice, I. C. (2005). Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 8245–8250. <https://doi.org/10.1073/pnas.0409902102>
- Van Dijk, H., Boudry, P., McCombre, H., & Vernet, P. (1997). Flowering time in wild beet (*Beta vulgaris* ssp. *maritima*) along a latitudinal cline. *Acta Oecologica*, 18, 47–60. [https://doi.org/10.1016/S1146-609X\(97\)80080-X](https://doi.org/10.1016/S1146-609X(97)80080-X)
- Van Dijk, H., & Hautekèete, N.-C. (2014). Evidence of genetic change in the flowering phenology of sea beets along a latitudinal cline within two decades. *Journal of Evolutionary Biology*, 27, 1572–1581. <https://doi.org/10.1111/jeb.12410>
- Varshney, R. K., Shi, C., Thudi, M., Mariac, C., Wallace, J., Qi, P., ... Xu, X. (2017). Pearl millet genome sequence provides a resource to improve agronomic traits in arid environments. *Nature Biotechnology*, 35, 969–976. <https://doi.org/10.1038/nbt.3943>
- Warnes, G. R., Bolker, B., Bonebakker, L., Gentleman, R., Liaw, W. H. A., Lumley, T., & Venables, B. (2013). *gplots: Various R programming tools for plotting data*. Retrieved from <http://cran.r-project.org/package=gplots>
- Wasser, S. K., Shedlock, A. M., Comstock, K., Ostrander, E. A., Mutayoba, B., & Stephens, M. (2004). Assigning African elephant DNA to geographic region of origin: Applications to the ivory trade. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 14847–14852. <https://doi.org/10.1073/pnas.0403170101>
- Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, 38, 1358.
- Weller, J. L., & Ortega, R. (2015). Genetic control of flowering time in legumes. *Frontiers in Plant Science*, 6, 1–13.
- Westgate, J. M. (1908). *Farmers' Bulletin 339. Alfalfa*. Washington, DC: United States Department of Agriculture.
- Wing, J. E. (1912). *Alfalfa farming in America*. Chicago, IL: Sanders Publishing Company. <https://doi.org/10.5962/bhl.title.56525>
- Winn, A. A., & Gross, K. L. (1993). Latitudinal variation in seed weight and flower number in *Prunella vulgaris*. *Oecologia*, 93, 55–62. <https://doi.org/10.1007/BF00321191>
- Woods, E. C., Hastings, A. P., Turley, N. E., Heard, S. B., & Agrawal, A. A. (2012). Adaptive geographical clines in the growth and defense of a native plant. *Ecological Monographs*, 82, 149–168. <https://doi.org/10.1890/11-1446.1>
- Young, N. D., Debelle, F., Oldroyd, G. E. D., Geurts, R., Cannon, S. B., Udvardi, M. K., ... Roe, B. A. (2011). The *Medicago* genome provides insight into the evolution of rhizobial symbioses. *Nature*, 480, 520–524. <https://doi.org/10.1038/nature10625>

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How to cite this article: Helliwell EE, Faber-Hammond J, Lopez ZC, et al. Rapid establishment of a flowering cline in *Medicago polymorpha* after invasion of North America. *Mol Ecol*. 2018;00:1–17. <https://doi.org/10.1111/mec.14898>