# Adaptation and colonization history affect the evolution of clines in two introduced species

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## Summary

• Phenotypic and genetic clines have long been synonymous with adaptive evolution. However, other processes (for example, migration, range expansion, invasion) may generate clines in traits or loci across geographical and environmental gradients. It is therefore important to distinguish between clines that represent adaptive evolution and those that result from selectively neutral demographic or genetic processes.

• We tested for the differentiation of phenotypic traits along environmental gradients using two species in the genus *Silene*, whilst statistically controlling for colonization history and founder effects. We sampled seed families from across the native and introduced ranges, genotyped individuals and estimated phenotypic differentiation in replicated common gardens.

• The results suggest that post-glacial expansion of *S. vulgaris* and *S. latifolia* involved both neutral and adaptive genetic differentiation (clines) of life history traits along major axes of environmental variation in Europe and North America. Phenotypic clines generally persisted when tested against the neutral expectation, although some clines disappeared (and one cline emerged) when the effects of genetic ancestry were statistically removed.

• Colonization history, estimated using genetic markers, is a useful null model for tests of adaptive trait divergence, especially during range expansion and invasion when selection and gene flow may not have reached equilibrium.

## Introduction

It has long been recognized that evolutionary change occurs by both adaptive and nonadaptive mechanisms (Gould & Lewontin, 1979). Accordingly, tests for adaptive divergence have become more rigorous, often involving comparisons of evolutionary rates or divergences relative to some neutral expectation (Lewontin & Krakauer, 1973; Kimura, 1980; Spitze, 1993; Vasemagi, 2006; Keller & Taylor, 2008).

Clines in allelic frequencies and quantitative traits along environmental gradients are often interpreted as evidence for adaptive evolution (Endler, 1977; Eanes, 1999). The near-ubiquity of clines in traits along gradients, such as latitude, has been used to identify major biogeographical trends, such as Bergmann's rule (Blackburn *et al.*, 1999) and Allen's rule (Scholander, 1955). Classic studies of clinal variation in plants have played a particularly noteworthy role in our understanding of local adaptation (Clausen *et al.*, 1948; Antonovics & Bradshaw, 1970), with plant populations frequently showing covariance between environmental gradients and phenology, morphology, physiology and life history (for example, Drezner, 2003; Stinchcombe *et al.*, 2004; Ingvarsson *et al.*, 2006; Savolainen *et al.*, 2007).

In the study of adaptation along a gradient, it is important to recognize and estimate other evolutionary processes that generate correlations between quantitative traits and geography or the environment. For example, the balance between genetic drift and migration can generate patterns of isolation by distance across geographical or environmental gradients that closely mimic adaptive clines (Vasemagi, 2006). Range expansion and colonization history can also leave a geographical signature of clinal variation through a series of successive founder events (Amsellem *et al.*, 2000; Clegg *et al.*, 2002), or when genetically distinct demes expand into a new range

(Hewitt, 2000). A classic example of such 'demic diffusion' comes from clinal gradients in allelic frequencies among European humans, a consequence of the rapid expansion of Neolithic farmers into Europe (Cavalli-Sforza *et al.*, 1993).

Similarly, clines may also arise during the colonization phase of a species invasion. The colonization history of many invasive species is accompanied by multiple introductions from different native range sources (Wares *et al.*, 2005; Dlugosch & Parker, 2008). Because founding events can generate genetic structure and deviations from the equilibrium balance between gene flow, drift and selection (Whitlock & McCauley, 1990, 1999; McCauley *et al.*, 1995), multiple introductions can quickly generate patterns that resemble isolation by distance or clinal variation when sources establish at different points of introduction (for example, Roman, 2006; Keller & Taylor, 2008). Numerous studies of species invasions have reported latitudinal clines for traits such as body size and mass, height, number of stems, flowering time, fecundity and physiology (Johnston & Selander, 1964; Weber & Schmid, 1998; Huey *et al.*, 2000; Kollmann & Banuelos, 2004; Maron *et al.*, 2004, 2007; Leger & Rice, 2007; Montague *et al.*, 2008). Although colonization history is expected to have a major influence on the population and quantitative genetics of introduced species, few studies have attempted to control for colonization history when analysing clines (but see Maron *et al.*, 2004). The implications are that the evolutionary causes of clinal patterns and, in particular, the importance of selection in generating clines are often unclear (Keller & Taylor, 2008).

*Silene vulgaris* and *Silene latifolia* (Caryophyllaceae) are weedy herbaceous plants that have a history of post-glacial expansion throughout Europe from divergent refugia and a recent invasion in North America (Baker, 1948; Taylor & Keller, 2007). The inferred routes of expansion in Europe are congruent with the post-glacial history of many European plants and animals (Hewitt, 2000; Petit *et al.*, 2003). In both *S. latifolia* and *S. vulgaris*, historical isolation and expansion coincided with phenotypic divergence, generating covariances between geography, climate, genotypes and phenotypes (Mastenbroek *et al.*, 1983; Keller, 2008). During the last 200 yr, both species have colonized North America and rapidly expanded their distributions. The *S. vulgaris* and *S. latifolia* systems are thus ideally suited for an empirical evaluation of the extent to which phenotypic clines during range expansion represent adaptive evolution vs demographic and neutral genetic processes.

In this study, we tested for genetic differentiation in phenotypic traits across environmental gradients whilst statistically controlling for selectively neutral processes that can also generate clines (for example, colonization history, gene flow and genetic drift). The approach builds on the tradition of estimating the genetic divergence of traits relative to neutral loci ($F_{ST}$ vs $Q_{ST}$; Spitze, 1993), but is, in practice, more similar to methods that statistically account for background variation in genetic ancestry that frequently complicates genome-wide association studies (Price *et al.*, 2006). Specifically, we describe the multidimensional genotypic space of neutral loci using principal coordinates analysis (PCO), and remove the covariance between marker loci and phenotypes using multiple regression. Covariance between the residuals and geographical or environmental variables then provides an estimate of adaptive clinal variation in phenotype after the major axes of neutral genetic variation have been removed. We apply this approach to the post-glacial expansion and recent invasion history of *S. vulgaris* and *S. latifolia*, and show that adaptation and neutral/historical processes combine to generate clines in many aspects of the phenotype. In other traits, however, phenotypic trait variation is fully explainable by a null model of neutral genetic ancestry.

## Materials and Methods

*Silene vulgaris* (Moenche) Garcke (bladder campion) and *Silene latifolia* Poiret (white campion) are short-lived, perennial plants in the family Caryophyllaceae. *Silene vulgaris* is gynodioecious, with self-compatible hermaphrodites and a mixed-mating system (Glaettli *et al.*, 2006), whereas *S. latifolia* is dioecious. Both species have evolutionary histories of dynamic range expansion, becoming widespread following post-glacial expansion throughout their native range in Eurasia (Taylor & Keller, 2007). The species were first reported in North America in the early 1800s, probably introduced from Europe as contaminant in clover seed and waste from ocean-going ships that deposited their ballast (soil, sand, gravel, silage) in North American port cities (Martindale, 1876; Baker, 1948; McNeill, 1977). Both species are widespread weeds of disturbed areas in North America (McNeill, 1977; Randall, 2002), where their ranges extend over much of the northern USA and southern Canada, and southwards in the eastern USA at higher elevations.

Seed capsules (maternal families) were collected from both species throughout Europe and North America as part of an ongoing series of experiments focused on elucidating the nature and evolution of invasiveness. We then combined a population genetic analysis of neutral variation with common garden experiments to measure life history traits that impact on fitness and are likely to experience variation in the direction and intensity of selection in response to regionally varying climate. These included size and growth-related traits that reflect the capacity for light capture and carbon fixation (height, number of stems, number of leaves), phenological traits that reflect the schedule of life history events (germination time, time to flowering) and reproductive traits that reflect effort expended towards producing sexual progeny (number of flowers, weeks spent flowering, number of fruits). Because the functional values of these traits often vary with environmental conditions, especially temperature, precipitation and photoperiod, they have been used extensively in

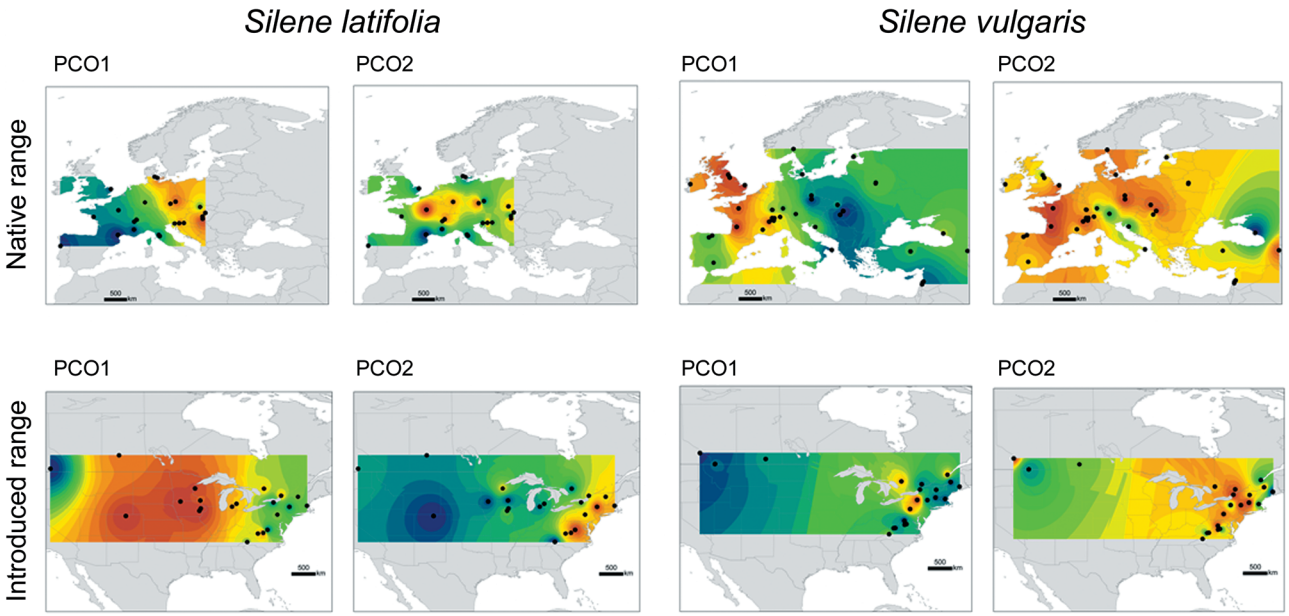


**Fig. 1** The geographical distribution of genotyped samples of *Silene latifolia* and *Silene vulgaris* in Europe and North America. Colours represent contours in genetic space using marker loci (interpolated using the inverse distance weighting method), with panels showing the first two principal coordinates (PCO1 and PCO2) obtained from amplified fragment length polymorphism (AFLP) genotyping. In all plots, cooler colours represent higher PCO scores and warmer colours represent lower PCO scores. The scaling of colour is identical across continents (within species) to facilitate interpretation of the introduction history.

previous studies of clines in plants (for example, Kollmann & Banuelos, 2004; Maron *et al.*, 2004; Stinchcombe *et al.*, 2004; Etterson *et al.*, 2008; Samis *et al.*, 2008).

Replicated experiments at different garden sites are essential when estimating evolved differences among ranges (for example, Williams *et al.*, 2008). Accordingly, the experimental design for each species involved one garden planted in Ontario, Canada and one garden established in Virginia, USA. These sites span most of the latitudinal breadth of each species in eastern North America. However, the two sites within Ontario and within Virginia were not identical, and the design and traits measured were different for each species, and so the two sets of gardens are treated as different experiments.

## Common garden experiments

For the *S. vulgaris* experiment, maternal families were selected from 36 European and 23 North American localities (Fig. 1). Open-pollinated seed capsules were collected from up to seven maternal plants in each location. In all, 100 families from North America and 100 families from Europe were grown in two gardens: a fallow field in Ontario, Canada (45.8642°N, –79.4362°W) and a hay pasture in Virginia, USA (37.8577°N, –78.8208°W). There were *S. vulgaris* populations in the vicinity of each site (~1 km away), but not in the fields themselves.

Six weeks before planting, seeds were surface sown in a randomized design into plug trays filled with a standard potting mix. Plants were germinated in growth chambers under misted conditions. The chambers were set to a diurnal photoperiod of 12 h : 12 h (light : dark) and a temperature cycle of 21 and 12°C, and trays were rotated daily within the chambers to reduce position effects. Germination was scored daily and four seedlings per family were randomly chosen for planting into the field (total $N = 2$ sites $\times$ 2 continents of origin $\times$ 100 families/continent $\times$ 4 plants/family = 1600 plants).

Seedlings were transplanted into the field during spring of 2005. The gardens consisted of four rectangular subplots, each containing 200 plants (one from each family). Seedlings were watered, and those that were lost as a result of transplant mortality < 2 wk post-planting were replaced with a seedling from the same family at the same stage of growth. The existing vegetation was cut back periodically at each site to avoid over-competition.

Weekly censuses were conducted throughout the 2005 growing season and again in 2006 to assay performance and measure traits important to plant fitness. During the first census in which a plant was observed flowering, we measured the height of the tallest stem (cm), the number of primary stems, the number of leaves > 1 cm long and the number of flowers. During each subsequent census, plants were checked for reproductive status (vegetative or flowering) and the number of new flowers and mature fruits.

The *S. latifolia* field data are a subset of the data collected from a larger experiment involving common gardens established in Europe and North America. These garden sites included plots that excluded natural enemies with pesticides and control plots. For the current study, we only used data from the control plots in the North American sites. Sixty full sib families were grown in old fields near Queen's University Biological Station in Ontario, Canada (44.5788°N, –76.3804°W) and Mountain Lake Biological Station (37.3603°N, –80.5527°W).

The 60 families were generated using seed collections from 30 European and 30 North American populations. A sample of seeds was grown from two maternal families (capsules) from each population, and a male and a female each from separate families were crossed to yield one full sib family per population. The seeds were sown into a standard potting mix in plastic pots (diameter, 2.5 cm) (trade name 'conetainers'). Seeds were germinated on a mist bench, thinned to single plants and grown to rosettes under ambient conditions in the glasshouse. Germination was poor in some families, leaving us with 28 full sib families from Europe and 30 full sib families from North America.

The sites were planted in May 2006. Plants were cut back to 1 cm of growth above the root crown. The subset of the plots used in this study involved six plants per family (360 plants) in each of the two sites. The sites were divided into 12 subplots with an equal number of North American and European families in each. For planting, the soil plug was extracted from the conetainer and inserted into a small disturbance created with a 15-cm auger at 50-cm intervals. Plants were censused in the spring (late April to early May), summer (late July to early August) and autumn (mid to late October) for a total of five censuses over two seasons. At each census, we recorded the number of basal and stem leaves, heights of all stems to the nearest centimetre, number of flowers open or in bud and the number of fruits. We also calculated the reproductive speed of each plant as the inverse of the census at which we first observed a plant to be reproductive (five for the first census, one for the most recent, zero if never found to be reproductive).

## Amplified fragment length polymorphism (AFLP) genotyping

We attempted to genotype one representative from each family in each species (~260 individuals overall) for genetic analysis. However, after losses caused by incomplete germination and mortality, we were able to obtain samples for molecular work from 175 *S. vulgaris* families (92 European, 83 North American) and 44 *S. latifolia* families (22 European, 22 North American). Genomic DNA was isolated from leaf tissue using Qiagen DNeasy plant miniprep kits. Plants were genotyped for AFLP loci following the methods described elsewhere (Keller, 2008). Briefly, we used an Applied Biosystems (Foster City, CA, USA) Plant Mapping Kit in which 50–100 ng of genomic DNA was digested at room temperature overnight with a pair of restriction enzymes (*Eco*RI and *Mse*I; New England Biolabs, Ipswich, MA, USA) and ligated to double-stranded adapters in a single 10-μl reaction. Preselective and selective amplifications were performed in 10-μl reaction volumes using a touchdown polymerase chain reaction (PCR) protocol suggested by the manufacturer (Applied Biosystems Plant Mapping Kit). We used four primer pair combinations in *S. vulgaris* [two 5-FAM-labelled pairs (*Eco*RI: ACA/*Mse*I: CTC, *Eco*RI: ACA/*Mse*I: CAC) and two TAMRA-labelled pairs (*Eco*RI: ACC/*Mse*I: CAG, *Eco*RI: AGC/*Mse*I: CTG)] and three primer pairs in *S. latifolia* [one 5-FAM-labelled pair (*Eco*RI: ACA/*Mse*I: CAC), one TAMRA-labelled pair (*Eco*RI: ACC/*Mse*I: CAG) and one JOE-labelled pair (*Eco*RI: AGG/*Mse*I: CAA)]. Selective amplification products were sized relative to the ROX-500 standard using Genemapper v4.0 software (Applied Biosystems) on an ABI 3130xl sequencer. All samples were normalized to the sum of the sample signal within Genemapper, calculated across the entire project. Polymorphic peaks > 50 relative fluorescence units (RFUs) were scored within a size range of 100–400 bp (*S. vulgaris*) or 50–400 bp (*S. latifolia*). Automated scoring was manually verified and peak presence/absence was converted to binary (0/1) coding for analysis. In total, 267 polymorphic AFLP loci were scored for *S. vulgaris* and 133 for *S. latifolia*.

## Data analysis

We performed an initial analysis to remove variance attributable to site and plot effects using repeated measures analysis of variance (ANOVA) (PROC MIXED: SAS, 2004). For the *S. vulgaris* data, garden site and year were treated as fixed effects, whereas plot within each site was a random effect; individual plants were the repeated subject effects. Traits were either natural logarithmically transformed (time to germination, time to flowering, number of leaves at flowering, number of flowers, flowering period, number of fruits) or square root transformed (height, number of stems) before analysis to improve normality and homoscedasticity. For *S. latifolia*, traits were either logarithmically transformed (basal leaves, stem leaves, number of flowers, number of fruits) or square root transformed (average stem height, number of stems). Residuals from these ANOVAs were exported for further analysis.

We estimated the genetic similarity among AFLP genotypes using the first two eigenvectors from PCO to ordinate one individual from each family in continuous multilocus genotype space. Principal coordinates were computed on the binary AFLP data matrix using R Package v4.0 (http://www.bio.umontreal.ca/Casgrain/en/labo/R/v4/index.html). Genetic distances among individuals were defined as 1 – Jaccard's similarity index $J_{ij}$, which calculates the similarity based on

shared bands among individuals and ignores shared absences [$J_{ij} = a/(a + b + c)$, where $a$ is the number of bands shared by individuals $i$ and $j$, $b$ is the number of bands in $i$ but not $j$, and $c$ is the number of bands in $j$ but not $i$]. We then combined each family's scores from the first two PCO axes with the phenotypic data (residuals from the above ANOVAs). To avoid pseudoreplicating the PCO results among individuals within sibships, we used family means for the phenotypic data, and hereafter treat families as the units of observation in all subsequent analyses.

We associated each family with the latitude from which it was collected, as well as climate-based environmental variables. Environmental variables were obtained from the WorldClim dataset of interpolated global climate (Hijmans *et al.*, 2005), and we focused specifically on eight bioclimatic ('Bioclim') variables derived from long-term observations of temperature and precipitation (1950–90) mapped to a resolution of 5 arc-min (0.083°). The eight variables were the mean annual temperature, minimum temperature of the coldest month, maximum temperature of the warmest month, temperature seasonality (SD across months × 100), mean annual precipitation, mean precipitation during the driest month, mean precipitation during the warmest quarter year and precipitation seasonality (CV). We chose these eight variables because they represent more biologically meaningful descriptors of the conditions that plants experience during the growing season and overwintering relative to annual means. We extracted values for each climate variable for each of our sites using a geographical information system (GIS) and pooled observations across species and continents. We then summarized the major trends in climate using a principal components analysis (PCA) to reduce the dimensionality of the environmental data (PROC PRINCOMP: SAS, 2004). Spearman rank correlation was used to assess correlations within each continent between geography (latitude, longitude), climate (Prin1, Prin2) and neutral genetic variation (PCO1, PCO2). To visually portray how climate and ancestry varied geographically, we mapped the variation in PCA and PCO interpolated using the inverse distance weighting method (Chang, 2008).

We tested for phenotypic diversification along genetic and environmental clines within Europe and North America using general linear models estimated using restricted maximum likelihood (PROC MIXED: SAS, 2004). Although the models did not contain random effects, we chose to use PROC MIXED for its maximum likelihood estimation, which permits the calculation of measures of model fitting to the data. Accordingly, we adopted a model selection approach, using the Akaike information criterion ($AIC_c$), to assess the fit among three different candidate models (Johnson & Omland, 2004). In the first model (climate-only, or 'C'), we regressed each phenotypic trait on the first two PCA axes (Prin1 and Prin2) to test for clines with climate. In the second model (ancestry-only, or 'A'), we regressed traits on the first two PCO axes (PCO1 and PCO2) from the AFLP data to detect phenotypic associations with neutral loci that might have accumulated as a result of drift, migration, founder effects and other selectively neutral aspects of colonization history. Finally, we tested for phenotypic clines against the null expectation of ancestry by including as predictor variables the climate variables (Prin1 and Prin2) and the neutral genetic variables (PCO1 and PCO2) in the same model (climate–ancestry, or 'CA'). This last model was designed to test for clines with environmental variables that persist after controlling for nonadaptive processes that may have generated clines at neutral loci. It should be noted that the use of PCO to control for history assumes selective neutrality of AFLP loci; thus, any linkage disequilibrium between AFLPs and nearby loci under selection may remove some non-neutral variance. For each trait, we retained the model with the lowest $AIC_c$ score, and reported the difference in fit ($\Delta AIC_c$) between the best and next best models (Johnson & Omland, 2004). We also replicated the $AIC_c$ model selection analysis using latitude instead of the WorldClim variables for comparison with other studies that traditionally report latitudinal clines.

Summarizing climate variation with PCA is useful because it avoids statistical problems of collinearity and multiple testing. However, there is no guarantee that the PCA axes represent the combination of environmental variables to which the plants are adapting. Therefore, as a complementary approach to model selection using PCA, we also used stepwise multiple regression to build models that contained the set of individual climate variables and PCO axes that best explained the phenotypic variance.

## Results

### Associations among climate, geography and genetic ancestry

The eight WorldClim climate variables were summarized by the first two principal components (Prin1 and Prin2), which collectively explained 69% of the total variance (Table 1). These axes describe major climate trends on each continent: large values of Prin1 are cool and wet, with low precipitation

**Table 1** Principal components analysis of eight climate variables from the WorldClim dataset

| | Prin1 | Prin2 |
|---|---|---|
| Mean annual temperature | –0.26057 | 0.43095 |
| Minimum temperature of coldest month | –0.27154 | 0.55096 |
| Maximum temperature of warmest month | –0.10822 | –0.08102 |
| Temperature seasonality | 0.19115 | –0.52623 |
| Mean annual precipitation | 0.37404 | 0.30430 |
| Precipitation driest month | 0.49674 | 0.28180 |
| Precipitation warmest quarter | 0.51655 | 0.01367 |
| Precipitation seasonality | –0.39578 | –0.23463 |

**Table 2** Spearman rank correlation coefficients between latitude/longitude, climate and genetic ancestry

| | Europe | | North America | |
|---|---|---|---|---|
| | Latitude | Longitude | Latitude | Longitude |
| WorldClim variables: | | | | |
| Prin1 | 0.32195* | –0.11862 | –0.12117 | 0.81901**** |
| Prin2 | –0.21453 | –0.62254**** | –0.77199**** | 0.40126** |
| *Silene latifolia* AFLP: | | | | |
| PCO1 | –0.47134* | –0.79447**** | 0.02428 | 0.56748** |
| PCO2 | –0.18874 | –0.14229 | 0.17335 | –0.64201** |
| *Silene vulgaris* AFLP: | | | | |
| PCO1 | –0.27129** | 0.54292**** | 0.06259 | –0.28222** |
| PCO2 | –0.30230** | 0.21518* | 0.20545* | –0.22477* |

Prin1 and Prin2 are the first two eigenvectors from a principal components analysis of eight climate variables obtained from the WorldClim dataset. PCO1 and PCO2 are the first and second eigenvectors from a principal coordinates analysis of multilocus amplified fragment length polymorphism (AFLP). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

seasonality; small values are warm and dry, with more seasonal precipitation (Table 1). In Europe, this climate axis correlated positively with latitude, whereas, in North America, it correlated positively with longitude (Table 2; Fig. S1, see Supporting Information). For Prin2, large values are warm and wet, with low temperature and precipitation seasonality; small values are cold, dry and more seasonal (Table 1). In Europe, Prin2 correlated negatively with longitude, whereas, in North America, Prin2 correlated negatively with latitude and positively with longitude (Table 2; Fig. S1).

Neutral genetic diversity also showed strong geographical gradients on both continents (Fig. 1). In the native range of Europe, demic diffusion during the process of post-glacial expansion was evident as a strong east–west transition zone in central Europe for PCO1 in both species. This pattern generated a highly significant correlation of PCO1 with longitude (both species $P < 0.0001$, Table 2). Variation in PCO2 was more species specific: *S. latifolia* showed a genetically distinct region in central Europe, whereas, for *S. vulgaris*, there was evidence of a cohesive group between the southern Balkans and the area around the Black Sea. The invasion history into North America generated a more patchy spatial distribution of genetic diversity in both species. Nevertheless, the establishment points of the introduced genotypes and subsequent range expansions left significant correlations among PCO axes and geography, particularly for longitude (Table 2). Relating the distribution of PCO variation for both species between the native and introduced ranges suggests multiple introductions from eastern and western Europe (Fig. 1). In *S. latifolia*, most genotypes in eastern North America share PCO space with genotypes from central Europe (especially areas of eastern France and Germany), whereas genotypes around the Great Lakes and further west show greater affinity with genotypes from eastern Europe. In *S. vulgaris*, genotypes from eastern Europe are found throughout most of the introduced range, whereas western European genotypes are established locally in Pennsylvania, Ontario and Quebec.

## Clinal phenotypic evolution

Phenotypic clines with climate and ancestry were apparent in both species and on both continents (Tables 3, 4). In *S. vulgaris*, most traits were best fitted by models that combined climate and ancestry (12 of 16 models; Table 3). The importance of both selective and neutral processes governing genetically based phenotypic variation was indicated by the large number of significant regression coefficients for both climate of origin ($P < 0.05$ for 17 of 24 estimates for Prin1 and Prin2, collectively) and genetic ancestry ($P < 0.05$ for 13 of 32 estimates for PCO1 and PCO2, collectively).

In Europe, plants from cool, wet climates with low seasonality (large Prin1) were larger (produced more leaves and stems), spent more weeks flowering and produced more flowers and fruits than plants from warmer, drier climates (Table 3). Plants originating from warm, wet environments with low seasonality (large Prin2) were smaller (shorter height and fewer leaves), spent fewer weeks flowering and produced fewer fruits than plants from colder, drier, more seasonal climates (Table 3). Altogether, plants originating from cooler climates (large Prin1, small Prin2) exhibited greater allocation to vegetative growth and reproductive effort than did plants from warmer climates. Clines predicted by latitude and the stepwise selection of climate variables were generally congruent with model selection using PCA. Plants were larger and had greater reproductive effort at higher latitudes and cooler temperatures (Tables S1, S3, see Supporting Information). Effects of precipitation were more variable and depended on the timing of precipitation during the year. Plants from sites with abundant precipitation during the warmest quarter

**Table 3** Evolution of clines in life history traits with climate in *Silene vulgaris*

| | Best model | $\Delta AIC_c$ | Prin1 β | Simple model | Prin2 β | Simple model | PCO1 β | PCO2 β |
|---|---|---|---|---|---|---|---|---|
| Europe | | | | | | | | |
| Germination time | A | 7.6 | | | | | –0.5399 | –0.1737 |
| Number of leaves | CA | 8.8 | **0.1027****** | ↑ | –0.1512* | | –1.7189** | –1.2979 |
| Number of stems | CA | 5.9 | 0.0819** | ↑ | –0.0703 | | –0.7699 | –0.5664 |
| Stem height | CA | 4.9 | 0.0254 | | **–0.2347***** | ↓ | –0.0077 | –1.3003† |
| Time to flowering | A | 26.2 | | | | | 0.0097 | –0.1797* |
| Weeks flowering | CA | 8.8 | **0.0759****** | ↑ | –0.1057* | ↓ | –0.2144 | –0.9750† |
| Number of flowers | CA | 10.7 | **0.1478***** | ↑ | –0.1835 | ↓ | 0.3481 | –2.4726* |
| Number of fruits | CA | 11.4 | 0.1275** | ↑ | –0.4667** | ↓ | –2.3501* | –1.8814 |
| North America | | | | | | | | |
| Germination time | CA | 4.8 | 0.03738 | | –0.06503** | ↓ | **–1.079****** | 1.0858** |
| Number of leaves | CA | 1.7 | 0.0500 | | 0.0868* | ↑ | –0.2534 | –1.1983* |
| Number of stems | A | 3.8 | | ↑ | | | –0.7003† | –0.8398 |
| Stem height | CA | 6.0 | –0.0076 | | **2.005****** | ↑ | 0.5665 | –1.0013† |
| Time to flowering | A | 22.7 | | | | ↓ | –0.0016 | –0.0376 |
| Weeks flowering | CA | 11.4 | 0.1401** | ↑ | **0.0953***** | ↑ | 0.8637** | **–1.7084***** |
| Number of flowers | CA | 16.3 | 0.2877** | ↑ | 0.1633* | ↑ | 1.9851** | **–3.4684***** |
| Number of fruits | CA | 4.2 | **0.4104****** | ↑ | 0.0808 | | 0.7688 | 0.0797 |

Best models were chosen among three candidate models [C (climate), A (ancestry) and CA (climate and ancestry)] based on the lowest $AIC_c$ score. $\Delta AIC_c$ reports the difference in fit between the best and next best models. Regression coefficients (β) from best models are given for climate (Prin1 and 2) and ancestry (PCO1 and 2) effects. Large values of Prin1 represent cool, wet climates, whereas large values of Prin2 represent warm, wet climates with low seasonality. Arrows show direction of clines significant under a simple (C only) model. Bold values are significant after sequential Bonferroni correction ($\alpha = 0.05$). †, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

**Table 4** Evolution of clines in life history traits with climate in *Silene latifolia*

| | Best model | $\Delta AIC_c$ | Prin1 β | Simple model | Prin2 β | Simple model | PCO1 β | PCO2 β |
|---|---|---|---|---|---|---|---|---|
| Europe | | | | | | | | |
| Basal rosette leaves | A | 7.5 | | | | | –0.0539 | 0.5664 |
| Stem leaves | A | 5.2 | | | | ↑ | –0.8591† | –0.2703 |
| Number of stems | A | 3.0 | | | | ↑ | –0.2487 | –0.0388 |
| Stem height | CA | 0.5 | –0.2743* | ↓ | 0.1804* | ↑ | 0.5875 | 0.7545 |
| Reproductive speed | A | 1.5 | | | | ↑ | –2.5177* | –3.2692* |
| Number of flowers | A | 6.2 | | | | | –0.7720 | –0.1272 |
| Number of fruits | A | 8.7 | | ↑ | | | 0.5962* | –0.0230 |
| North America | | | | | | | | |
| Basal rosette leaves | A | 8.4 | | ↑ | | | 0.5814† | 0.3507 |
| Stem leaves | A | 9.1 | | ↑ | | | 0.3218 | 0.2664 |
| Number of stems | A | 8.5 | | | | | 0.0701 | 0.1222 |
| Stem height | A | 1.4 | | | | | –0.1066 | –0.2221 |
| Reproductive speed | A | 4.3 | | | | | –1.7919 | –0.3949 |
| Number of flowers | A | 7.0 | | | | | –0.2777 | 0.7940† |
| Number of fruits | A | 7.9 | | | | | 0.2020 | 0.6180 |

Best models were chosen among three candidate models [C (climate), A (ancestry) and CA (climate and ancestry)] based on the lowest $AIC_c$ score. $\Delta AIC_c$ reports the difference in fit between the best and next best models. Regression coefficients (β) from best models are given for climate (Prin1 and 2) and ancestry (PCO1 and 2) effects. Large values of Prin1 represent cool, wet climates, whereas large values of Prin2 represent warm, wet climates with low seasonality. Arrows show direction of clines significant under a simple (C only) model. Bold values are significant after sequential Bonferroni correction ($\alpha = 0.05$). †, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

produced more leaves, flowered earlier and made more flowers, whereas plants from sites with high precipitation during the rest of the year produced fewer leaves and flowers (Table S3).

Strong clines linked to climate have also evolved very recently since the invasion of North America. Plants originating from sites with wet climates (large Prin1, small Prin2) germinated more rapidly, grew taller with more leaves and produced

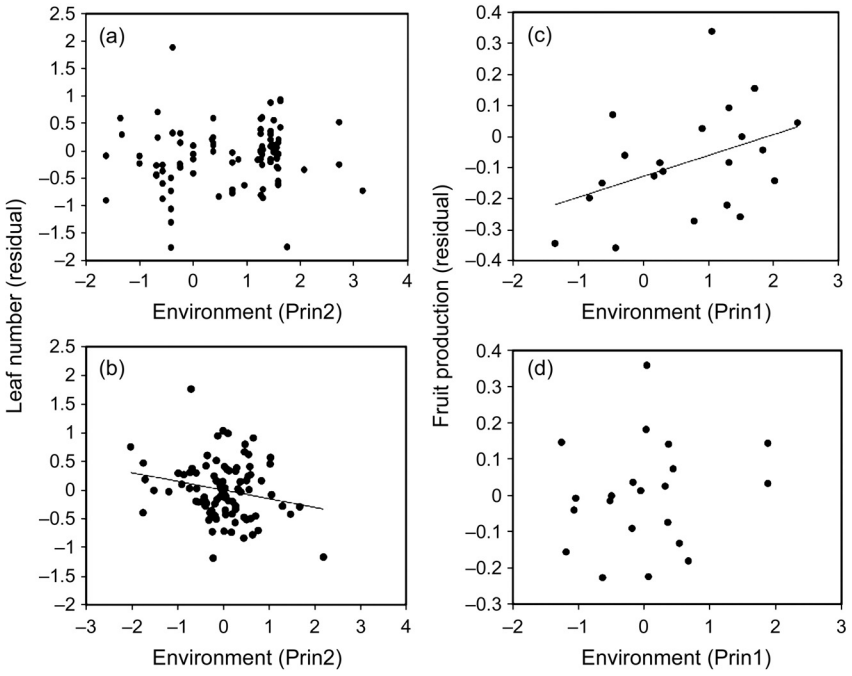


**Fig. 2** Interpretation of phenotypic clines along environmental gradients which change on inclusion of genetic ancestry. Panels show the relationship between leaf number and environment in *Silene vulgaris* (a, b), and the relationship between fruit number and environment in *Silene latifolia* (c, d). (a, c) Phenotypic clines using uncorrected data; (b, d) partial correlations after controlling for the effects of genetic ancestry (PCO1 and PCO2) in the model. Regression lines are shown for the significant relationships (see also Tables 3 and 4).

more flowers and fruits over a longer reproductive period than plants from drier climates (Table 3). Interestingly, reversals between continents in the direction of clines were observed, especially for the warm/wet vs cold/dry climate axis described by Prin2 (Table 3). Reversals in clines were also evident for latitude (four of five clines significant in both ranges reversed; Table S1). North American plants grew smaller and produced fewer flowers when they were from high latitudes, in contrast with plants from Europe. Similarly, stepwise models often included different sets of significant climate predictors in North America relative to Europe (Table S3). When the same climate variable was predictive of a trait in both ranges, the direction of the cline was always reversed (three of three clines reversed).

The inference of clines in *S. vulgaris* under a simple climate-only model sometimes changed dramatically with the inclusion of ancestry (Table 3). Neutral genetic ancestry among *S. vulgaris* genotypes was associated with several phenotypic traits in Europe and North America (Table 3). Accounting for ancestry revealed an environmental cline in leaf number that was not previously evident (Fig. 2). European plants originating from cold, dry sites (small Prin2) had significantly greater leaf production ($P = 0.0434$), but this effect would not have been observed without controlling for ancestry (C model: $P = 0.4016$). Likewise, multiple traits showing clinal variation under a climate-only model became nonsignificant or were better fitted by a model including ancestry (Table 3). A striking example was flower production among European plants. Under a climate-only model, flower number showed a highly significant relationship with Prin2 ($P = 0.0071$), which disappeared once ancestry was taken into account ($P = 0.1173$). Similar results were observed among North American plants for the number of stems and time to flowering, where ancestry-only models provided a better fit to the data than did models that included clinal variation with climate (Table 3).

In contrast with *S. vulgaris*, most traits in *S. latifolia* were best explained by a simple ancestry model (13 of 14 models; Table 4). Stem height was an exception, showing significant phenotypic clines in Europe after controlling for ancestry. Taller plants originated from environments that experienced greater precipitation than environments that produced smaller plants (small Prin1, large Prin2). Similar trends were observed with latitude, with most traits explained by ancestry-only models (Table S2, see Supporting Information). Stepwise regression revealed some influence of climate on phenotypic clines, particularly temperature seasonality (Table S4, see Supporting Information). Plants from more seasonal regions were larger (taller with more stem leaves) and faster to reproduce, but made fewer fruits overall. However, none of these relationships remained significant after Bonferroni correction. Clines with climate were generally absent from North America, except for basal leaves, which showed a positive relationship with temperature during the coldest month of the year (Table S4).

In multiple instances, phenotypic clines that were evident in *S. latifolia* under climate-only models were no longer supported after including ancestry (Table 4). For example, among European plants, there was a significant positive relationship between fruit production and Prin1 ($P = 0.0350$) under a climate-only model. However, an ancestry-only model provided a substantially better fit to the data ($\Delta AIC_c = 8.7$; Fig. 2). Specifically, higher fruit production occurred among

genotypes with large PCO1 scores that partially overlapped with the distribution of cool, wet climates in western and central Europe (Figs 1, S1). An ancestry-only model also provided a better fit to clines for stems, stem leaves and reproductive speed ($\Delta AIC_c$ from 1.5 to 9.1 for each trait; Table 4).

## Discussion

### Climate adaptation of *Silene* in Europe and North America

The history of post-glacial expansion and invasion in *S. latifolia* and *S. vulgaris* involved the differentiation of life history traits across geographically varying environments. Because family means for traits were observed across multiple common garden sites, these correlations are probably genetically based. In *S. latifolia*, variation in most traits was best explained by the genetic ancestry of families. In *S. vulgaris*, much of the phenotypic divergence occurred along the major axes of temperature and precipitation variation across Europe and North America. As most of these clines were significant after controlling for genetic ancestry, divergence in *S. vulgaris* probably reflects an evolutionary response to selection imposed by regional climate conditions.

Clines in size and growth traits often paralleled clines in reproductive traits. In Europe, *S. vulgaris* families from Mediterranean climates (hot, dry summers) were smaller and reproduced less than families from more temperate regions of northern Europe. In North America, families from the wetter eastern half of the continent were larger and had higher reproductive output than families from drier sites in western North America. Although tradeoffs between growth and reproduction are expected when resource acquisition is fixed, variance in resource acquisition leads to the evolution of positive correlations between growth and reproduction (Stearns, 1989). One hypothesis is that genotypes from Mediterranean Europe and western North America occupy marginal regions of the fundamental niche, and have evolved stress tolerance (for example, to heat and water availability) that limits the physiological function achievable under more permissive conditions. Ecological niche models for *S. vulgaris* suggest that this may be the case (S. Keller & D. Taylor, unpublished).

The evolution of phenotypic clines among North American *S. vulgaris* contributes to growing evidence that evolution can be pronounced and rapid following invasion (Blair & Wolfe, 2004; Lavergne & Molofsky, 2007; Facon *et al.*, 2008). The establishment of clines is a feature of many species invasions and has been interpreted as a hallmark example of adaptive evolution in action (Johnston & Selander, 1964; Lacey, 1988; Weber & Schmid, 1998; Huey *et al.*, 2000; Kollmann & Banuelos, 2004; Maron *et al.*, 2004, 2007; Leger & Rice, 2007; Etterson *et al.*, 2008; Montague *et al.*, 2008). In our study, divergence was often observed in excess of the neutral expectation generated by ancestry, offering particularly strong evidence that the clines are adaptive. Complementary approaches, such as reciprocal transplant experiments and functional assays, would help to further the case for the adaptive nature of the clines, as well as help to reveal their mechanistic basis.

Clinal variation is often expressed as correlation between phenotypes and environmental variation or geographical variation that covaries with environment (for example, latitude or altitude). We observed clines with climate and, as expected, climate covaried with geography to generate geographical clines as well. Interestingly, environmental variation often varied in opposite geographical directions on the two continents (Table 2), resulting in a clear reversal of the phenotypic clines documented in our study. Reversals of cline direction were especially evident along the Prin2 climate axis (Table 3) and latitude (Table S1). This pattern might have been misinterpreted to reflect different colonization histories had we not corrected for genetic ancestry, as the PCO axes showed significant correlations with latitude and longitude that differed in sign between ranges (Table 2). However, most clines maintained their reversal in direction, even after correcting for ancestry (Fig. 3), pointing towards differences in climate-imposed selection occurring within each range. This result is not wholly surprising, given that most of North America is characterized by a continental climate, whereas much of western and central Europe is moderated by the influence of the Gulf Stream (Menzel *et al.*, 2005). *Silene* in southern Europe (vicinity of the Mediterranean) often seems to restrict its flowering with the onset of summer heat, whereas flowering in North America starts late and can be truncated early whilst plants are still producing flowers by the onset of cold temperatures (S. R. Keller *et al.*, pers. obs.). These results suggest that unique patterns of selection may promote divergence between ranges as introduced populations adapt to novel climate conditions.

As seeds of *S. vulgaris* were field collected from open pollinated fruits, it is possible that some of the phenotypic variation observed could be attributable to maternal effects. Maternal effects in plants are principally manifested in early life history traits, especially seeds, and have diminishing influence beyond the seedling stage (Roach & Wulff, 1987). Only one of the traits measured in *S. vulgaris*, germination time, is expressed early in the life history, whereas all other traits are properties of adult plants. However, the cline in germination time with Prin2, observed for North American *S. vulgaris*, could be influenced by the environment in which the seeds developed (Table 3), and should therefore be interpreted cautiously.

### Parsing adaptive and nonadaptive origins of clinal variation

Although it has long been appreciated that plant populations are capable of rapid adaptive evolution along strong

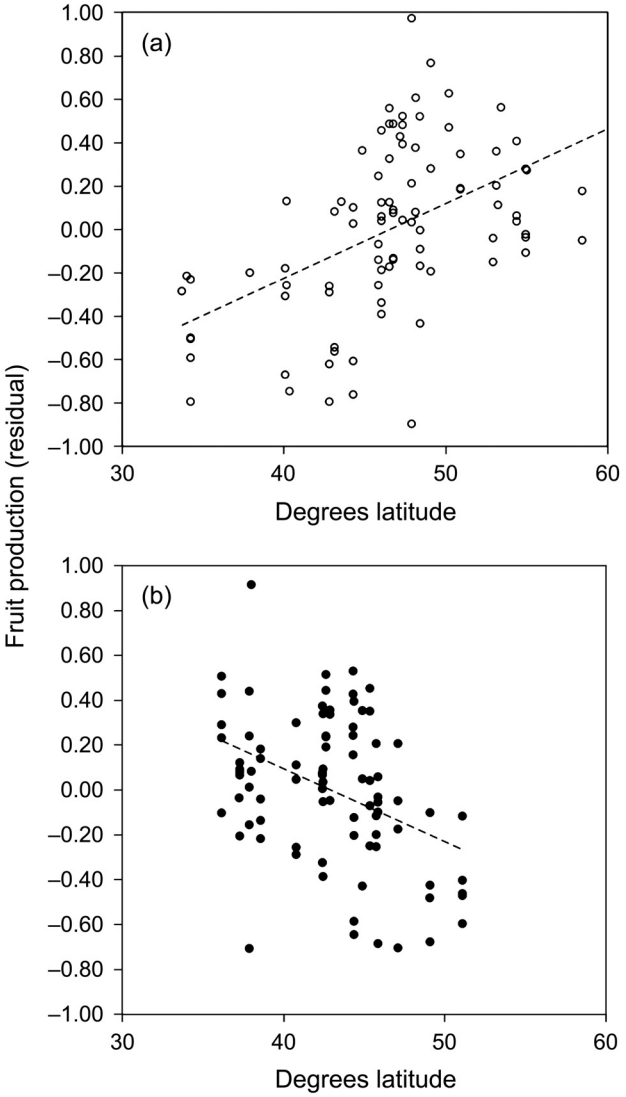


**Fig. 3** Reversal of latitudinal clines for fruit production of *Silene vulgaris* in Europe (a) vs North America (b). Fruit production is expressed as residuals from a model that removes the effects of genetic ancestry (PCO1 and PCO2).

environmental gradients (for example, Antonovics & Bradshaw, 1970), the novelty of our study comes from a broad consideration of the evolutionary processes that may act during non-equilibrium conditions, such as range expansion and invasion. Divergence during expansion will often reflect a history of spatially distinct dispersal and establishment, founder effects, genetic drift and gene flow. For this reason, recent studies on the evolution of clines are now beginning to incorporate null models based on genetic ancestry into tests for adaptation (Samis *et al.*, 2008). The effect of selectively neutral demographic processes on divergence is highly acute during species invasions. Invasions frequently involve multiple introductions of genetically distinct lineages sampled from different portions of the native range and established at different points within the introduced range (see reviews in Bossdorf *et al.*, 2005; Novak & Mack, 2005; Wares *et al.*, 2005; Roman & Darling, 2007; Dlugosch & Parker, 2008). The complexity inherent in many introduction histories means that controlling for ancestry effects is of utmost importance when testing for evolution in invasive species (Keller & Taylor, 2008).

We found strong correlations between neutral genetic variance and gradients in geography and climate in both the native and introduced ranges of *Silene*. Because neutral variance covaries with phenotype, and is nonrandomly distributed in space, ancestry affects how we interpret the causal processes affecting phenotypic clines. Our interpretation of adaptively evolved clines relied on the pairing of phenotypic and molecular data on the same family lines, which allowed us to test for phenotypic divergence relative to a neutral expectation (Keller & Taylor, 2008). A few previous studies of phenotypic clines during invasions recognized the need for the consideration of alternative mechanisms of divergence, such as colonization history (Lacey, 1988; Maron *et al.*, 2004; Montague *et al.*, 2008), but ours is the first to explicitly control for the influence of founding events, drift and migration in the generation of clines. Controlling for demographic processes significantly altered our interpretation of the processes of evolution in several cases. For some traits, significant clines could be fully accounted for by shared history at neutral loci (for example, number of flowers in European *S. vulgaris* and number of fruits in European *S. latifolia*; Tables 3, 4; Fig. 2). In other traits, accounting for demographic and historical effects revealed support for adaptive differentiation that was not otherwise evident (for example, number of leaves in European *S. vulgaris*; Fig. 2).

How conservative are these tests of clinal adaptation? It is possible for putatively neutral loci to diverge via hitchhiking when they are closely linked to loci under selection. Therefore, recent and/or strong selection could generate linkage disequilibrium that sets up clines even at neutral loci. Any effect of hitchhiking would tend to make our test for adaptation more conservative, as some of the putatively neutral variance controlled for may be linked to regions under selection. Our approach should be complementary to other methods of inferring robust evidence for clinal evolution, such as the observation of coincident parallel clines reported for several species invasions (Huey *et al.*, 2000; Leger & Rice, 2007; Maron *et al.*, 2007). Our method could be especially useful in this context, by controlling for colonization history that might otherwise obscure a parallel cline.

## Species differences in clinal differentiation

Phenotypic clines were observed more frequently in *S. vulgaris* than in *S. latifolia*. As the *S. vulgaris* experiment involved more families, part of this difference probably reflects differences in statistical power between the two field experiments. To explore this possibility, we used a power analysis to

estimate the likelihood of detecting significant phenotypic clines in *S. latifolia* relative to *S. vulgaris*. For each trait, we computed the partial correlation coefficient ($r_p$) with one climate Prin axis, whilst controlling for the other Prin axis and both PCO axes, and computed the power to reject a false null hypothesis based on the sample size in each species on each continent (PROC POWER: SAS, 2004). The mean effect size did not differ between species (mean absolute value of $r_p$: *S. latifolia*, 0.205; *S. vulgaris*, 0.239; $t = -0.994$; $P = 0.3242$). As expected, the difference in sample size between *S. latifolia* and *S. vulgaris* leads to differences in statistical power. Given the effect sizes observed, we had 4.4 times as much power to reject the null hypothesis in *S. vulgaris* relative to *S. latifolia.* However, we also note that our process of model selection relied only on how well different predictors explained the data. In this regard, C and A models should have been selected at about the same frequency, all else being equal, as they possessed the same sample sizes and number of parameters. Thus, the large number of traits favouring a neutral ancestry model in *S. latifolia* vs the predominance of models including environmental clines in *S. vulgaris* might reflect a genuine difference in biology between the species. It is also possible that the climate variation summarized by the PCA axes did not capture the selective gradients important to *S. latifolia*. When stepwise regression was used, there was more support for clines with climate, although none of these was significant after Bonferroni correction (Table S4). Interestingly, most of the evidence for phenotypic clines with climate was in Europe, with very few variables approaching significance in North America. Previous studies in *S. latifolia* have reported divergence in life history traits among European populations (Delph *et al.*, 2002; Wolfe *et al.*, 2004), and one trait (age at first flowering) has previously been shown to exhibit clinal variation with latitude (Jolivet & Bernasconi, 2007). There is also phenotypic divergence between Europe and North America consistent with adaptive evolution, although not expressly clinal in nature (Blair & Wolfe, 2004; Wolfe *et al.*, 2004). Thus, although there is some evidence that life history traits vary clinally in the native range, adaptive evolution along regional environmental gradients in the introduced range appears to be less prominent relative to neutral divergence or other selective processes. Of course, it is also possible that *S. latifolia* is adapting along environmental gradients in North America not included in these analyses.

### Conclusion

Phenotypic covariance between traits and the environment can arise from nonadaptive processes, and a complete understanding of adaptive differentiation – or lack thereof – requires that these processes are taken into account. We observed clines in the native and introduced ranges of two weedy plants that have a global history of post-glacial expansion and invasion. Many of these clines were robust to the inclusion of information on genetic ancestry, strongly supporting the inference of adaptive evolution within each range in response to regionally varying climate, whereas other clines were consistent with neutral genetic processes. Studies of phenotypic evolution during range expansions, species invasions and other situations characterized by nonequilibrium demographic and genetic conditions will benefit from the incorporation of a neutral expectation into tests of evolutionary change.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Fig. S1** Distribution of WorldClim climate variation for *Silene latifolia* and *Silene vulgaris* sites in Europe and North America.

**Table S1** Evolution of clines in life history traits with latitude in *Silene vulgaris*

**Table S2** Evolution of clines in life history traits with latitude in *Silene latifolia*

**Table S3** Stepwise regression of phenotypic clines predicted by ancestry (principal coordinates analyses, PCOs) and climate for *Silene vulgaris*

**Table S4** Stepwise regression of phenotypic clines predicted by ancestry (principal coordinates analyses, PCOs) and climate for *Silene latifolia*

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Table S1. Evolution of clines in life history traits with latitude in *Silene vulgaris*.

| | Best Model | $\Delta AIC_c$ | Latitude β | Simple model | PCO1 β | PCO2 β |
|---|---|---|---|---|---|---|
| Europe | | | | | | |
| Germination time | A | 8.0 | | | -0.5399 | -0.1737 |
| Number of leaves | LA | 3.4 | $0.0508^{****}$ | ↑ | -0.5086 | -0.3553 |
| Number of stems | A | 1.0 | | ↑ | -0.7339 | -0.5563 |
| Stem height | A | 4.1 | | | $-1.2984^{**}$ | $-1.9686^{\dagger *}$ |
| Time to flowering | L | 3.1 | $0.0047^{****}$ | ↑ | | |
| Weeks flowering | LA | 9.0 | $0.0374^{****}$ | ↑ | $0.6231^{\dagger}$ | -0.1218 |
| Number of flowers | LA | 12.9 | $0.0723^{****}$ | ↑ | $1.7930^{*}$ | -0.7148 |
| Number of fruits | LA | 2.4 | $0.0540^{**}$ | ↑ | 0.8177 | -1.4829 |
| North America | | | | | | |
| Germination time | A | 6.5 | | | $-1.079^{****}$ | $1.0175^{**}$ |
| Number of leaves | A | 1.5 | | ↓ | -0.3377 | $-1.3517^{*}$ |
| Number of stems | A | 7.3 | | | $-0.7003^{\dagger}$ | -0.8398 |
| Stem height | LA | 6.3 | $-0.0625^{***}$ | ↓ | $0.7267^{\dagger}$ | -0.7424 |
| Time to flowering | LA | 7.3 | $0.0022^{*}$ | ↑ | -0.0061 | -0.0576 |
| Weeks flowering | LA | 7.1 | $-0.0336^{****}$ | ↓ | $0.7051^{*}$ | $-1.8042^{****}$ |
| Number of flowers | LA | 3.8 | $-0.0607^{**}$ | ↓ | $1.6228^{*}$ | $-3.6797^{***}$ |
| Number of fruits | A | 2.9 | | [↓] | 0.1678 | -0.4768 |

Best models were chosen among three candidate models: L (latitude), A (ancestry), and LA (latitude and ancestry) based on the lowest $AIC_c$ score. $\Delta AIC_c$ reports the difference in fit between the best and next best models. Regression coefficients (β) from best models are given for climate (Prin1 and 2) and ancestry (PCO1 and 2) effects. Arrows show direction of clines significant under a simple (C only) model. $^{\dagger}P<0.10$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$

Table S2. Evolution of clines in life history traits with latitude in *Silene latifolia* (see footnote in Table S1 for details).

| | Best Model | $\Delta AIC_c$ | Latitude β | Simple model | PCO1 β | PCO2 β |
|---|---|---|---|---|---|---|
| Europe | | | | | | |
| Basal rosette leaves | A | 5.2 | | | -0.0539 | 0.5663 |
| Stem leaves | A | 3.3 | | | -0.8591$^{\dagger}$ | -0.2611 |
| Number of stems | A | 5.0 | | | -0.2487 | -0.0388 |
| Stem height | A | 4.9 | | | -0.6172$^{\dagger}$ | 0.1380 |
| Reproductive speed | A | 1.5 | | | -2.5177$^{*}$ | -3.2692$^{*}$ |
| Number of flowers | A | 5.1 | | | -0.7722 | -0.1272 |
| Number of fruits | A | 5.4 | | | 0.5962$^{*}$ | -0.0230 |
| North America | | | | | | |
| Basal rosette leaves | A | 6.2 | | | 0.5814$^{\dagger}$ | 0.3507 |
| Stem leaves | A | 3.4 | | | 0.3218 | 0.2664 |
| Number of stems | L | 1.3 | | [↓] | | |
| Stem height | A | 0.2 | | | 0.3766 | 0.2344 |
| Reproductive speed | A | 4.3 | | | -1.7919 | -0.3949 |
| Number of flowers | A | 7.0 | | | -0.2777 | 0.7940$^{\dagger}$ |
| Number of fruits | A | 6.3 | | | 0.2020 | 0.6180 |

$^{\dagger}P<0.10$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$

Table S3. Stepwise multiple regression estimates of phenotypic clines predicted by ancestry (PCOs) and climate for *Silene vulgaris*.

| Europe | | | | | North America | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Model** | **$R^2$** | **β** | ***F*** | ***P*** | **Model** | **$R^2$** | **β** | ***F*** | ***P*** |
| *Germination time* | 0.024 | | | | | 0.412 | | | |
| PCO1 | | -0.53838 | 2.17 | 0.1438 | PCO1 | | -1.07523 | 23.95 | **<0.0001** |
| | | | | | PCO2 | | 1.20229 | 13.22 | **0.0005** |
| | | | | | AVGANNTMP | | 0.00446 | 3.55 | 0.0633 |
| | | | | | MINTMPCLDMNTH | | -0.00513 | 8.48 | 0.0047 |
| *Number of leaves* | 0.343 | | | | | 0.182 | | | |
| PCO1 | | -1.21141 | 3.98 | 0.0493 | PCO2 | | -1.03467 | 3.62 | 0.0609 |
| AVGANNTMP | | -0.00785 | 8.53 | 0.0045 | AVGANNPCP | | 0.00048 | 3.55 | 0.0631 |
| PCPDRYMNTH | | -0.01253 | 10.21 | **0.0020** | MAXTMPWRMMNTH | | 0.00333 | 2.97 | 0.0891 |
| PCPSEASON | | -0.01142 | 10.31 | **0.0019** | | | | | |
| PCPWRMQTR | | 0.00165 | 2.65 | 0.1071 | | | | | |
| *Number of stems* | 0.171 | | | | | 0.102 | | | |
| MAXTMPWRMMNTH | | -0.00557 | 18.10 | **<0.0001** | AVGANNPCP | | 0.00055 | 5.27 | 0.0244 |
| | | | | | TMPSEASON | | 0.00009 | 4.67 | 0.0339 |
| *Stem height* | 0.330 | | | | | 0.283 | | | |
| AVGANNPCP | | -0.00076 | 29.04 | **<0.0001** | PCO2 | | -1.16050 | 3.85 | 0.0534 |
| MINTMPCLDMNTH | | -0.00651 | 24.39 | **<0.0001** | MINTMPCLDMNTH | | 0.00575 | 26.05 | **<0.0001** |
| *Time to flowering* | 0.302 | | | | | 0.074 | | | |
| AVGANNTMP | | 0.00227 | 12.93 | **0.0005** | MINTMPCLDMNTH | | -0.00024 | 6.27 | 0.0143 |
| PCPSEASON | | -0.00125 | 15.73 | **0.0002** | | | | | |
| PCPWRMQTR | | -0.00012 | 2.45 | 0.1212 | | | | | |
| TMPSEASON | | 0.00005 | 27.97 | **<0.0001** | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MAXTMPWRMMNTH | | -0.00208 | 18.59 | **<0.0001** | | | | | |
| *Weeks flowering* | 0.344 | | | | | 0.472 | | | |
| AVGANNPCP | | -0.00060 | 20.42 | **<0.0001** | PCO1 | | 0.99331 | 12.37 | **0.0007** |
| PCPWRMQTR | | 0.00250 | 45.58 | **<0.0001** | PCO2 | | -1.38248 | 10.21 | **0.0020** |
| | | | | | AVGANNPCP | | 0.00166 | 9.61 | 0.0027 |
| | | | | | PCPDRYMNTH | | -0.01016 | 2.54 | 0.1151 |
| | | | | | MAXTMPWRMMNTH | | 0.00355 | 4.82 | 0.0312 |
| *Number of flowers* | 0.323 | | | | | 0.375 | | | |
| PCO2 | | -2.26626 | 4.24 | 0.0427 | PCO1 | | 2.07806 | 10.33 | **0.0019** |
| PCPDRYMNTH | | -0.01785 | 13.05 | **0.0005** | PCO2 | | -3.23411 | 11.16 | **0.0013** |
| PCPWRMQTR | | 0.00670 | 28.62 | **<0.0001** | AVGANNPCP | | 0.00191 | 19.96 | **<0.0001** |
| *Number of fruits* | 0.275 | | | | | 0.203 | | | |
| AVGANNTMP | | -0.01891 | 27.11 | **<0.0001** | AVGANNPCP | | 0.00193 | 18.77 | **<0.0001** |
| AVGANNPCP | | -0.00139 | 20.29 | **<0.0001** | TMPSEASON | | 0.00012 | 2.66 | 0.1070 |

The *P*-value to enter or exit the model was set to 0.15. Bold values are significant after sequential Bonferroni correction. Worldclim climate variables: AVGANNTMP (average annual temperature), MAXTMPWRMMNTH (maximum temperature during the warmest month of the year), MINTMPCLDMNTH (minimum temperature during the coldest month of the year), TMPSEASON (temperature seasonality), AVGANNPCP (average annual precipitation), PCPDRYMNTH (average precipitation during the driest month of the year), PCPWRMQTR (average precipitation during the warmest quarter of the year), PCPSEASON (precipitation seasonality).

Table S4. Stepwise multiple regression estimates of phenotypic clines predicted by ancestry (PCOs) and climate for *Silene latifolia*.

| Europe | | | | | North America | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Model** | $R^2$ | **β** | ***F*** | ***P*** | **Model** | $R^2$ | **B** | ***F*** | ***P*** |
| *Basal rosette leaves* | 0.251 | | | | | 0.385 | | | |
| PCO2 | | 0.67632 | 4.75 | 0.0421 | PCPWRMQTR | | -0.00095 | 2.49 | 0.1323 |
| PCPSEASON | | -0.00745 | 3.01 | 0.099 | TMPSEASON | | 0.00014 | 4.92 | 0.0396 |
| | | | | | MINTMPCLDMNTH | | 0.00575 | 8.87 | 0.0081 |
| *Stem leaves* | 0.427 | | | | | 0.146 | | | |
| PCPWRMQTR | | 0.00199 | 3.64 | 0.0727 | MINTMPCLDMNTH | | 0.00108 | 3.43 | 0.0789 |
| TMPSEASON | | 0.00031 | 10.08 | 0.0052 | | | | | |
| MINTMPCLDMNTH | | 0.00851 | 5.83 | 0.0266 | | | | | |
| *Number of stems* | 0.414 | | | | | 0.107 | | | |
| PCO1 | | -0.46767 | 6.82 | 0.0177 | AVGANNTMP | | 0.00106 | 2.4 | 0.1368 |
| AVGANNTMP | | 0.00264 | 3.91 | 0.0636 | | | | | |
| PCPDRYMNTH | | 0.00415 | 9.19 | 0.0072 | | | | | |
| *Stem height* | 0.394 | | | | | 0.146 | | | |
| PCO1 | | 1.18025 | 2.32 | 0.1447 | AVGANNPCP | | 0.00076 | 3.42 | 0.0793 |
| PCPSEASON | | 0.01731 | 4.53 | 0.0474 | | | | | |
| TMPSEASON | | 0.00034 | 8.81 | 0.0082 | | | | | |
| *Reproductive speed* | 0.629 | | | | | 0.124 | | | |
| PCO2 | | -3.39764 | 11.14 | 0.0037 | PCO1 | | -1.65399 | 2.84 | 0.1075 |
| AVGANNTMP | | 0.01409 | 3.24 | 0.0888 | | | | | |
| TMPSEASON | | 0.00061 | 14.27 | 0.0014 | | | | | |
| *Number of flowers* | 0.259 | | | | | 0.343 | | | |
| PCPSEASON | | 0.01523 | 5.43 | 0.0309 | PCO2 | | 1.38544 | 9.93 | 0.0053 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| PCPWRMQTR | | 0.00169 | 2.89 | 0.1054 | AVGANNPCP | 0.00032 | 2.85 | 0.1079 |
| *Number of fruits* | 0.274 | | | | | | | |
| TMPSEASON | | -0.00011 | 7.18 | 0.0148 | (none) | | | |

See legend for Table S3 for details.

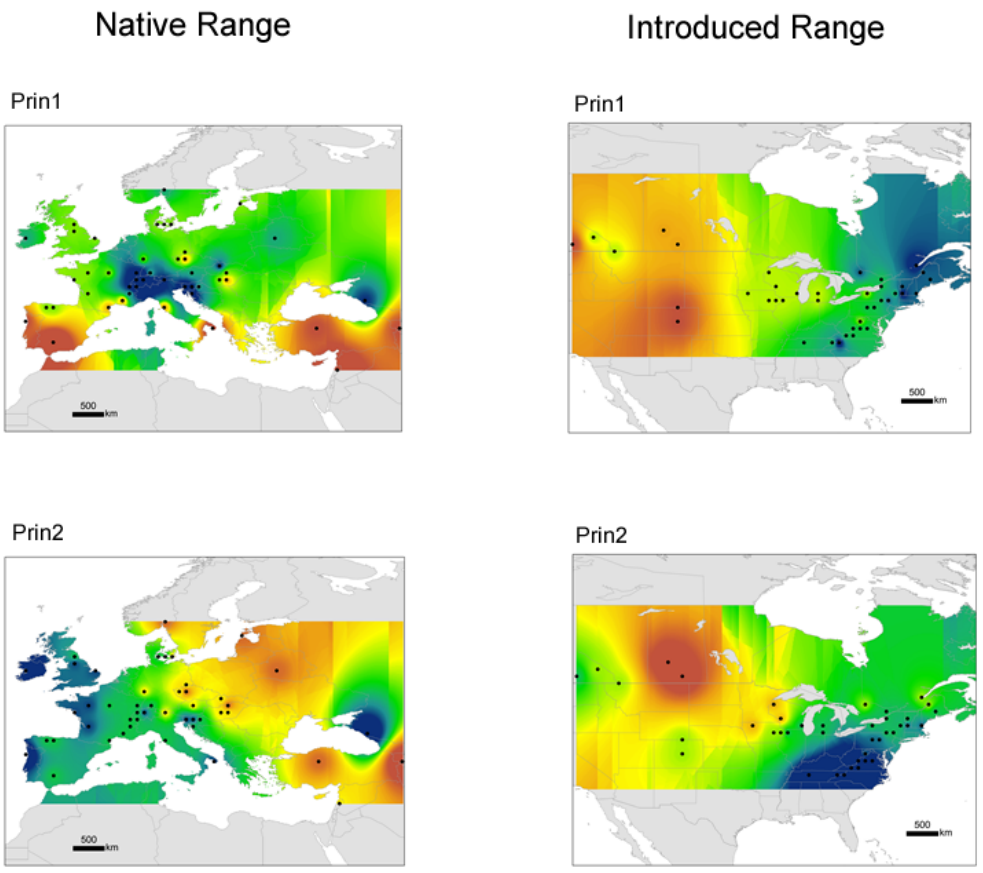


Fig. S1. Distribution of WorldClim climate variation for *S. latifolia* and *S. vulgaris* sites in Europe and North America. Colors represent interpolated contours in climate (inverse distance weighting method) as summarized by the first two principal components (Prin1 and Prin2) obtained from eight WorldClim climate variables. Black circles are locations of *S. latifolia* and *S. vulgaris* used this study. Cooler colors correspond to larger values of Prin1 and Prin2; warmer colors correspond to smaller values.