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MULTIVARIATE ADAPTATION BUT NO INCREASE IN COMPETITIVE ABILITY IN INVASIVE *GERANIUM CAROLINIANUM* L. (GERANIACEAE)

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Adaptive evolution can affect the successful establishment of invasive species, but changes in selective pressures, loss of genetic variation in relevant traits, and/or altered trait correlations can make adaptation difficult to predict. We used a common-garden experiment to assess trait correlations and patterns of adaptation in the invasive plant, *Geranium carolinianum*, sampled across 20 populations in its native (United States) and invasive (China) ranges. We used multivariate $Q_{ST} - F_{ST}$ tests to determine if phenotypic differences between countries are attributable to adaptation. We also compared population-level variation within each country to assess whether local adaptation resulted in similar multivariate phenotypes in the United States and China. Between countries, most phenotypic differences are indistinguishable from genetic drift, although we detected a signature of adaptation to the colder, drier winters in China. There was no evidence for increases in invasive traits in China. Within countries, strong multivariate adaptation appears to be driven by latitudinal climatic variation in the United States, but not in China. Additionally, adaptive trait combinations as well as their underlying correlations differ between the two countries, indicating that adaptation in invasive populations does not parallel patterns in native populations due to differences in selection pressures, genetic constraints, or both.

KEY WORDS: Common garden, F_{ST}, G matrix, local adaptation, Q_{ST}.

Invasion biology has long focused on the ecological impacts of species invasions. However, as rapid evolution has been demonstrated both in invading populations and recipient communities, there has been increased focus on the evolutionary consequences of invasions. The ability of a nonindigenous species to respond to natural selection within its new range will likely play a large role in successful establishment, range expansion, and extent of its ecological impacts (Sakai et al. 2001; Lee 2002). Evolutionary potential in traits that contribute to invasiveness, such as growth and fecundity, are also necessary starting points for evolutionary hypotheses of invasions (e.g., Blossey and Notzold 1995).

Evolutionary potential is determined by the availability of genetic variation in traits targeted by selection. Although founder effects, as measured by neutral marker diversity, are commonly associated with species introductions (Novak and Mack 2005; Wares et al. 2005; Dlugosch and Parker 2008a), genetic variance for quantitative traits may not be affected in the same way as neutral markers due to their complex genetic architecture (Neiman and Linksvayer 2005). Because adaptive potential is ultimately tied to heritable variation, it is necessary to assess both neutral genetic and quantitative trait variation to fully understand the evolutionary consequences of plant invasions.

Heritable variation in phenotypic traits, measured using quantitative genetic methods (Lynch and Walsh 1998), can be compared to neutral genetic markers as a test of adaptation among populations and regions (Spitze 1993; Merila and Crnokrak 2001; Whitlock and Gilbert 2012). In this framework, known as $Q_{ST} - F_{ST}$, population differentiation in a quantitative trait (Q_{ST}) is compared to the null expectation of differentiation by genetic

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drift as measured by neutral markers (F_{ST}). If $Q_{ST} < F_{ST}$, the trait is more similar across populations than expected due to drift, indicating evidence of selection for a uniform trait value across all populations (unifying selection). If $Q_{ST} = F_{ST}$, trait differentiation is indistinguishable from the effects of drift, and there is no evidence for selection. Finally, if $Q_{ST} > F_{ST}$, populations are more genetically differentiated than expected, which is evidence for local adaptation to a heterogeneous landscape.

Importantly, because Q_{ST} is a measure of population divergence over past evolutionary history, $Q_{ST} - F_{ST}$ is a test of response to selection, rather than selection itself. Response to selection is dependent on the availability of heritable genetic variation, but is also affected by correlations among phenotypic traits, which can arise from a number of sources, such as pleiotropy, linkage, and selection. These correlations are summarized by the genetic covariance matrix **G** and can restrict, promote, or have no effect on phenotypic evolution (Lande and Arnold 1983). Thus, a multivariate perspective that simultaneously considers many traits is necessary to account for such correlations, as well as the fact that natural selection often acts on trait combinations rather than individual traits. Multivariate versions of the $Q_{ST} - F_{ST}$ test that incorporate trait correlations (Chenoweth and Blows 2008; Martin et al. 2008) allow explicit tests of adaptive divergence in a multivariate framework.

Adaptive evolution over short timescales is well-documented in invasive species, even with severe founder effects (Reznick and Ghalambor 2001; Maron et al. 2004; Phillips et al. 2006; Colautti and Barrett 2010; Xu et al. 2010; Chun et al. 2011), but few studies (e.g., Franks et al. 2012) have examined multivariate adaptation across ranges. Different evolutionary trajectories between ranges could result from changes in biotic interactions (e.g., as predicted by the evolution of increased competitive ability, or EICA, hypothesis; Blossey and Notzold 1995) or other selective pressures, or as a result of geographic variation in genetic constraints (e.g., **G** matrix variation).

Previous work on the annual plant *Geranium carolinianum* using neutral genetic markers (Shirk et al. 2014) demonstrated that invasive populations in China experienced mild founder effects during colonization and range expansion. We use a common-garden study including native (United States) and invasive (Chinese) populations, and tests of multivariate adaptation to address the following questions: Are phenotypic differences between the invasive and native ranges the result of adaptive evolution? Have Chinese populations evolved traits associated with increased invasiveness? Are patterns of local adaptation similar within each country, or do adaptive trait combinations differ in the United States and China? Additionally, we investigate climate as a possible driver of adaptive evolution both between and within countries.

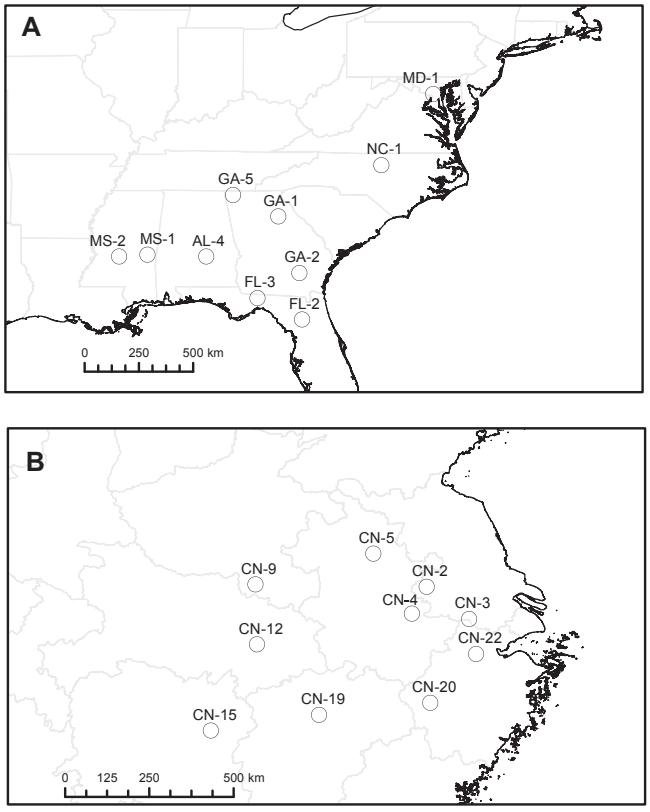

Figure 1. Map of *Geranium carolinianum* populations sampled in (a) the native range (southeastern United States) and (b) the invasive range (eastern China). Population labels correspond to those in Shirk et al. (2014).

Methods

STUDY SPECIES

The native range of *G. carolinianum* extends throughout the continental United States and north into Canada (Aedo 2000). In the southeastern United States, it is a common weed of fields, lawns, and roadsides. It is naturalized in the eastern plains region of China, where it has been present for approximately 90 years and grows in similar habitats as its native range. Genetic marker data indicate multiple introductions and a moderate rangewide reduction in neutral genetic diversity as a result of founder effects during range expansion (Shirk et al. 2014). As a winter annual, *G. carolinianum* germinates in the fall, overwinters as a rosette, and bolts and flowers in the spring. Flowers are small, insect-pollinated, and self-compatible, although populations tend to range from mixed-mating to highly outcrossing (Shirk and Hamrick, 2014).

FIELD COLLECTIONS

Seeds were collected during the spring and summer of 2009 from 10 native populations across the southeastern United States and 10 invasive populations across eastern China (Fig. 1). Population

sizes ranged from >50 to several thousand individuals. A minimum of 24 reproductive individuals per population were selected haphazardly and all ripe fruits were collected from each plant. Seeds were cleaned from their fruits, pooled by maternal family, and stored in paper envelopes at room temperature until use.

COMMON GARDEN AND TRAIT MEASUREMENTS

To test for adaptive evolution, we measured traits related to growth, reproduction, and physiology. Growth and reproductive traits should be closely linked to fitness, and physiological traits can be used to characterize resource-use strategies (Wright et al. 2004). We measured 13 traits at the rosette (hereafter juvenile) and reproductive (hereafter adult) stages, split into three trait categories to facilitate quantitative genetic analysis: juvenile, adult, and physiological. Juvenile traits consisted of juvenile establishment time (used as a proxy for juvenile growth rate), juvenile rosette size, and specific leaf area (SLA). Adult traits consisted of adult rosette size (rosette size at reproduction), days to first flower, height, reproductive ratio (aboveground biomass allocation to reproduction), aboveground biomass, and seed weight (mass of 10 seeds). Physiological traits were measured on juvenile plants and consisted of leaf nitrogen content (N_{mass}) and carbon isotope ratio ($\delta^{13}C$, a proxy for integrated leaf water-use efficiency). We also measured root biomass and root mass ratio (RMR) for a subset of individuals.

Seven seeds from 10 maternal families from all 20 populations (10 invasive and 10 native; $n = 1400$) were germinated from January 3–8, 2012. To promote uniform germination, seeds were nicked with a razor blade and soaked in water for two days before planting. Germinating seeds were planted in 10 × 10 × 10 cm pots filled with a pine bark potting mix and placed in a greenhouse without supplemental lighting. Pots were divided into seven blocks in a randomized complete block design to account for environmental variation in the greenhouse. All seeds for a block were started on the same day, so that all plants within a block were the same age. All stage-based measurements (e.g., date of first flower) were corrected for differences in germination time prior to analysis.

Juvenile establishment time was estimated from planting to full expansion of the third rosette leaf. This growth stage was arbitrarily chosen as an estimate of the seedling to rosette transition. Length of the fully expanded third leaf (including the petiole, approximately equivalent to rosette radius) was measured as an estimate of juvenile rosette size. Between February 21 and March 2, when plants were still in the juvenile rosette stage, the most recently fully expanded leaf (MRFEL) was harvested between 8 and 10 a.m. Leaves were placed in plastic bags on ice to maintain hydration, and scanned on a flat-bed scanner (Xerox Docu-Mate 632, Norwalk, CT) by mid-afternoon. Leaf area of the lamina was calculated from digital scans using ImageJ software (Abràmoff et al. 2004). Leaves were then dried at 60°C to a constant weight and weighed. SLA was calculated as leaf area/leaf mass ($mm^2\ mg^{-1}$). A subset of 600 dried MRFEL samples (10 populations per region, six families per population, five individuals per family) was ground to a fine powder in a borosilicate glass scintillation vial with a glass rod and analyzed for leaf N_{mass} and $\delta^{13}C$ (NA1500, Carlo Erba Strumentazione, Milan, Italy). All leaf traits were calculated using leaf lamina only, excluding the petiole (Cornelissen et al. 2003).

Plants were monitored daily for flowering. Adult rosette size was recorded as rosette diameter for each plant on its day of first flower. Each plant was harvested 28 days after its first flower, at which time plants were beginning to senesce but retained most of their seeds. At harvest, height was recorded and plants were divided into vegetative and reproductive biomass, dried to a constant weight at 60°C, and weighed. Reproductive ratio was calculated as reproductive biomass/total aboveground biomass ($g\ g^{-1}$). Additionally, 10 seeds (from two entire ripe fruits) were pooled and weighed to calculate seed weight for each plant. For one block (200 plants), roots were washed immediately after harvesting, dried at 60°C, and weighed. RMR was calculated as root biomass/total biomass ($g\ g^{-1}$).

There was an infestation of fungus gnats (species of *Orfelia* and/or *Bradyseia*) on roughly half of the experiment in mid-March (after MRFEL harvest but just before flowering), and larvae damaged developing leaf and inflorescence buds at the center of the rosette in affected plants. To control for the effect of herbivory, damage was quantified at harvest as the ratio (number of undamaged bolt stems)/(total number of bolt stems) for use as a covariate in statistical modeling.

NEUTRAL GENETIC DIVERSITY ANALYSIS

Between 23 and 52 seeds per population were germinated separately from the common garden plants and used for allozyme genotyping. Individual allozyme variants are occasionally reported to be under selection (e.g., Karl and Avise 1992; Johannesson et al. 1995), and it is possible that our panel of allozyme loci contains some alleles under selection. However, across species allozyme F_{ST} values appear to be strongly affected by characteristics that govern neutral processes, such as dispersal potential (Hamrick and Godt 1996). Thus, allozymes are generally consistent with selectively neutral expectations, and a sufficiently large panel of loci will, on average, provide a reasonable estimate of neutral genetic divergence. Additionally, we found concordant patterns of genetic structure in *G. carolinianum* using microsatellites and allozymes (Shirk et al. 2014), which supports the neutrality of our allozyme panel. In total, 782 individuals (362 from China and 420 from the United States) were genotyped at 28 allozyme loci. Locus information and buffers for allozyme extraction and gel electrophoresis can be found in the Supporting

Information. Multilocus variance components and *F*-statistics were calculated using the package *hierfstat* in R (Goudet 2004; R Development Core Team 2012).

VARIATION AND ADAPTATION IN UNIVARIATE TRAITS

Significant differences among regions and populations within regions for mean trait values were assessed with ANOVA, including region, population, and block as factors. Block was not included in the model for root biomass because data for this trait were collected from a single block (one individual per family, 10 families per population, 20 populations). Damage was included as a covariate for potentially affected traits: adult rosette size, height, days to first flower, and all biomass traits. Juvenile establishment time was log-transformed to achieve normality.

Quantitative trait differentiation between regions (Q_{CT}) and among populations within each region (United States Q_{SC}, and China Q_{SC}) were calculated as $Q_{CT} = V_{reg}/(V_{reg} + V_{pop} + 2V_{fam})$ and $Q_{SC} = V_{pop}/(V_{pop} + 2V_{fam})$, where V_{reg} is the among-region genetic variance, V_{pop} is the among-population genetic variance, and V_{fam} is the variance among families within populations, muliplied by four to account for half-sib families (Lynch and Walsh 1998). Because we used maternal families grown from field-collected seed, V_{fam} is an estimate of broad-sense heritability. Additionally, although we assumed that families consist of half-sibs, because the seeds were field collected, it is likely that the families were mixtures of full- and half-sibs. Due to these factors, our estimates of V_{fam} may be somewhat inflated, biasing our Q_{SC} and Q_{CT} estimates downward.

Variance components were calculated using restricted maximum likelihood (REML) with the *lme4* package in R (Bates et al. 2011). For Q_{CT}, the model included region, population nested within region, family nested within population, and block as random effects; for Q_{SC}, the model included population, family nested within population, and block as random effects. Damage was used as a random covariate for appropriate traits. Q_{SC} was not calculated for root biomass because there was no within-family replication and V_{fam} could not be modeled.

We used a simulation method based on parametric bootstrapping (Whitlock and Guillaume 2009) for the $Q_{CT} - F_{CT}$ and $Q_{SC} - F_{SC}$ comparisons. In contrast to earlier methods that estimate confidence intervals around empirical F_{ST} and Q_{ST} values, this approach generates a null distribution of Q_{ST} values for each trait assuming neutral trait evolution. Using this distribution, we calculated the probability that the observed Q_{ST} is a result of neutral evolution (the null hypothesis of $Q_{ST} = F_{ST}$). Note that because our study includes regional as well as population divisions, the traditional $Q_{ST} - F_{ST}$ comparison is termed $Q_{SC} - F_{SC}$ (i.e., divergence among populations within regions).

For each trait, the null distribution is generated by bootstrapping variance components (V_{fam} and V_{pop}) assuming neutrality, and calculating $Q_{SC} - F_{SC}$ for each iteration. Thus, the distribution is centered at $Q_{SC} - F_{SC} = 0$, and the quantile of observed $Q_{SC} - F_{SC}$ is used as the *P*-value for hypothesis testing. Quantiles from zero to 0.025 indicate divergent selection ($Q_{SC} > F_{SC}$, or $Q_{SC} - F_{SC} > 0$), and quantiles from 0.975 to 1.0 indicate uniform selection ($Q_{SC} < F_{SC}$, or $Q_{SC} - F_{SC} < 0$). The sampling distribution around V_{fam} is estimated using a χ^2 distribution with ($n_{pops} - 1$) × (n_{fam}) degrees of freedom (O'Hara and Merila 2005; Whitlock and Guillaume 2009). Under the assumption of neutrality, V_{pop} is estimated as

$$(2\bar{F}_{SC}V_{fam})/(1 - \bar{F}_{SC}),$$

with a sampling distribution estimated from a χ^2 distribution with ($n_{pops} - 1$) degrees of freedom (Whitlock 2008; Whitlock and Guillaume 2009). For each bootstrap iteration, F_{SC} is estimated by bootstrapping the allozyme data across loci, V_{fam} and V_{pop} are estimated by random draws from their respective sampling distributions, and a neutral Q_{SC} is calculated.

The null distribution for the regional test $Q_{CT} = F_{CT}$ was calculated similarly, with the addition that neutral V_{reg} was estimated as

$$(\bar{F}_{CT}V_{pop} + 2\bar{F}_{CT}V_{fam})/(1 - \bar{F}_{CT}),$$

and its sampling distribution as a χ^2 distribution with ($n_{reg} - 1$) degrees of freedom. Degrees of freedom associated with the V_{fam} and V_{pop} distributions were modified appropriately to accommodate inclusion of region in the model.

VARIATION AND ADAPTATION IN MULTIVARIATE TRAITS

Quantitative trait differentiation can be investigated in a multivariate framework to account for trait correlations. Multivariate population differentiation is estimated by the matrix $\mathbf{F_{SCq}}$, and multivariate regional differentiation is estimated by $\mathbf{F_{CTq}}$ (Kremer et al. 1997; Chenoweth and Blows 2008) as follows, where $\mathbf{G}$, $\mathbf{D}$, and $\mathbf{R}$ are within-population, among-population, and among-region covariance matrices, respectively:

$$\mathbf{F_{SCq}} = [\mathbf{D} + 2\mathbf{G}]^{-1/2}\mathbf{D}[\mathbf{D} + 2\mathbf{G}]^{-1/2}, \text{ and}$$

$$\mathbf{F_{CTq}} = [\mathbf{R} + \mathbf{D} + 2\mathbf{G}]^{-1/2}\mathbf{R}[\mathbf{R} + \mathbf{D} + 2\mathbf{G}]^{-1/2}.$$

Thus, the matrices $\mathbf{F_{SCq}}$ and $\mathbf{F_{CTq}}$ are the multivariate forms of Q_{SC} and Q_{CT}. To compare $\mathbf{F_{SCq}}$ and $\mathbf{F_{CTq}}$ with estimates of neutral population differentiation F_{SC} and F_{CT}, $\mathbf{F_{SCq}}$ and $\mathbf{F_{CTq}}$ are decomposed into their component eigenvectors and eigenvalues. Each eigenvector, $\mathbf{x_i}$, represents a linear combination of traits that describes population (or regional) multivariate trait

differentiation. The magnitude of differentiation along each eigenvector is the associated eigenvalue, λ_i, which ranges from zero to one. For the purposes of the $Q_{SC} - F_{SC}$ comparison, $\mathbf{x_i}$ is a "composite" trait that varies across populations and has a Q_{SC} of λ_i.

Traits were split into three groups for analysis: juvenile traits (juvenile establishment time, juvenile rosette size, and SLA), adult traits (days to first flower, adult rosette size, height, reproductive ratio, aboveground biomass, and seed weight), and physiological traits (leaf N_{mass} and $\delta^{13}C$). Physiological traits, although measured on juvenile plant tissue, were treated separately because they were measured on a subset of individuals. The remaining traits were split into "juvenile" and "adult" groupings because variance component estimation was poor when they were treated as a single group, likely because our sample size was too small to simultaneously estimate the number of parameters necessary for a large, nine-trait dataset.

Prior to analysis, juvenile establishment time was log-transformed, and all traits were scaled to a mean of zero and variance of one. Covariance matrices were estimated using REML in the program WOMBAT (Meyer 2007), with region, population nested within region, family nested within population, and block as random effects for the regional analysis ($\mathbf{F_{CTq}}$), and population, family nested within population, and block as random effects for the United States and China separately to assess populations within regions (United States and China $\mathbf{F_{SCq}}$). Because WOMBAT does not allow random covariates, we did not include damage when modeling adult traits. However, because the damage covariate had very minor effects on univariate Q_{SC} values, omission from the multivariate model should not affect our results. Q_{SC} or Q_{CT} (λ_i) for each composite trait was compared with F_{SC} or F_{CT} as in the univariate analysis. For the parametric bootstrapping for each λ_i, V_{fam} was estimated as $\mathbf{x_i^T[G]x_i}$, or the variance in $\mathbf{G}$ available for the composite trait. The final composite trait from each $\mathbf{F_{SCq}}$ matrix was discarded because it is necessarily orthogonal to all other axes and is not independent from the other traits. Thus, the $Q_{SC} - F_{SC}$ comparison is performed for eight independent composite traits across three trait groupings: two juvenile, five adult, and one physiological.

TRAIT COVARIANCE AND DIFFERENTIATION IN THE UNITED STATES VERSUS CHINA

Adaptive population differentiation is influenced both by selective pressures and underlying trait correlations. To determine whether trait relationships differ in native and invasive populations, we compared $\mathbf{G}$ matrices for each trait set—adult, juvenile, and physiological—between the United States and China in two ways. We first used common principal components analysis (CPCA) to test the extent to which the two matrices are similar in structure: equal, proportional, sharing principal components, or unrelated (Flury 1988; Phillips and Arnold 1999). We used both step-up and model-building approaches. In the step-up approach, each model is compared against the next lower model in the hierarchy. Akaike information criterion (AIC) scores are compared in the model-building approach.

Second, we calculated similarity between native and invasive populations in their major axis of genetic variation (the first eigenvector of $\mathbf{G}$, or $\boldsymbol{g}_{max}$). We measured similarity as the angle between eigenvectors, $\theta = \cos^{-1}[\boldsymbol{g}_{max,\,US}]^T[\boldsymbol{g}_{max,\,China}]$ (Schluter 1996). This similarity measure ranges from $\theta = 0$ (vectors equal; complete similarity) to $\theta = 90$ (vectors orthogonal; complete dissimilarity). We also performed this comparison for eigenvectors of $\mathbf{F_{SCq}}$ matrices under local adaptation ($Q_{SC} > F_{SC}$) to determine whether adaptive differentiation occurs along similar axes in native and invasive populations.

CLIMATE

We used seven bioclimatic variables extracted from WorldClim (Hijmans et al. 2005) to describe climate in each population: mean annual temperature (BIO1), isothermality (BIO3), temperature seasonality (BIO4), mean temperature of coldest quarter (BIO11), annual precipitation (BIO12), precipitation seasonality (BIO15), and precipitation of coldest quarter (BIO19). These were chosen because they describe the winter–spring growing season of *G. carolinianum* as well as mean climatic differences between the two countries. We initially used a principal components analysis, including all populations, to determine major climatic differences between countries. We then performed principal components analyses separately for each country to generate a composite climate axis for each region (United States climate PC1 and China climate PC1). To test for an association between climate and adaptation within each country, population mean values (calculated from family means) for univariate and composite traits carrying the signature of adaptive differentiation within countries ($Q_{SC} > F_{SC}$) were correlated with their respective climate axes.

Results

NEUTRAL GENETIC DIVERSITY

Of the 28 allozyme loci used for genotyping, 17 were polymorphic across both China and the United States, and an additional five were polymorphic only in the United States. Regional genetic differentiation between the United States and China was low ($F_{CT} = 0.067$), and within countries, genetic structure was higher in native populations (United States $F_{SC} = 0.196$) than invasive populations (China $F_{SC} = 0.082$). This is consistent with differentiation patterns found across a wider sampling of 16 U.S. and 24 Chinese populations (Shirk et al. 2014). Within-population diversity was moderate and ranged from $H_e = 0.131$ to 0.219

(mean = 0.180) in the United States and H_e = 0.107 to 0.179 (mean = 0.139) in China. Genetic diversity parameters for each population can be found in Shirk et al. (2014).

PATTERNS OF TRAIT DIFFERENTIATION BETWEEN REGIONS

ANOVAs for the univariate traits showed significant differences between the United States and China for all traits except SLA ($P < 0.05$ for adult rosette size and $P < 0.0001$ for all others). However, only juvenile rosette size and N_{mass} were locally adapted ($Q_{CT} > F_{CT}$; Table 1). On average, individuals from China had 30% smaller juvenile rosettes and 8% higher N_{mass} than U.S. individuals. Differentiation between the United States and China for all other traits was indistinguishable from values expected for genetic drift, although it should be noted that it is statistically impossible to achieve a significant $Q_{CT} < F_{CT}$ due to the low levels of neutral divergence and a small sample size ($n = 2$ regions). This is evidenced by SLA and adult rosette size that had no genetic differentiation between regions ($V_{reg} = 0.000$) and a Q_{CT} of zero, but the statistical comparison with F_{CT} was not significant for either trait.

Of the eight composite traits resulting from the regional multivariate analyses, one adult trait and the physiological trait had a significant $Q_{CT} > F_{CT}$, indicating adaptive divergence between countries (Table 2). The adult composite trait, which explained 76% of total variation, had $Q_{CT} = 0.287$ and was largely described by days to first flower, height, and total biomass. The physiological composite trait had $Q_{CT} = 0.485$ and was affected almost entirely by N_{mass}. Chinese individuals are later flowering, taller, and have higher leaf N content but less aboveground biomass than U.S. individuals. Lack of a significant $Q_{CT} - F_{CT}$ for the juvenile composite traits was inconsistent with the univariate analysis, which showed strong regional differentiation for juvenile rosette size as a result of local adaptation.

Root biomass was not significantly different between countries (ANOVA; $P = 0.48$), but China had a significantly higher RMR than the United States (China RMR = 0.188; United States RMR = 0.158; $P < 0.0001$). Because root biomass and RMR were only obtained for one individual per family, we were unable to calculate Q_{CT} for these traits.

PATTERNS OF TRAIT DIFFERENTIATION WITHIN CHINA

Within China, mean population values for all univariate traits except reproductive ratio varied significantly (ANOVA, $P = 0.001$ for adult rosette size and $P < 0.0001$ for all other traits). Both RMR and root biomass differed significantly across populations as well ($P \leq 0.0001$).

Lack of differentiation for reproductive ratio was a result of unifying selection across populations ($Q_{SC} = 0.000$, $P < 0.01$; Table 3a). Additionally, five traits (juvenile establishment time, juvenile rosette size, SLA, N_{mass}, and aboveground biomass) were locally adapted across China ($Q_{SC} > F_{SC}$, Table 2a). Mean univariate Q_{SC} across all traits in China was 0.236.

In the multivariate analysis, five composite traits (both juvenile, two adult, and the physiological trait) had a significant $Q_{SC} > F_{SC}$, supporting local adaptation, and one adult trait had $Q_{SC} < F_{SC}$, supporting unifying selection (Table 4). Q_{SC} was close to one for composite traits representing the primary axes of differentiation, indicating strong multivariate differentiation: $Q_{SC} = 0.988$ for the primary juvenile trait, which was heavily affected by SLA; 0.987 for the primary adult trait, influenced by adult rosette size and aboveground biomass; and 0.990 for the physiological trait, which was nearly equally affected by N_{mass} and $\delta^{13}C$. Composite traits representing the secondary axes of differentiation for juvenile and adult traits also had a significant $Q_{SC} > F_{SC}$, but with much lower Q_{SC} values (0.182 for the juvenile trait and 0.319 for the adult trait).

PATTERNS OF TRAIT DIFFERENTIATION WITHIN THE UNITED STATES

Within the United States, all univariate traits, including RMR and root biomass, varied significantly among populations (ANOVA, $P \leq 0.001$ for SLA and juvenile establishment time; $P < 0.0001$ for all other traits). Three traits (juvenile rosette size, N_{mass}, and aboveground biomass) were locally adapted across populations, and no traits were under unifying selection (Table 3b). Average Q_{SC} across traits in the United States was 0.307, higher than the average Q_{SC} in China.

Four multivariate traits in the United States had a significant $Q_{SC} > F_{SC}$ (one juvenile, two adult, and the physiological trait), indicating local adaptation, and no traits had evidence for unifying selection (Table 5). The juvenile composite trait showing local adaptation ($Q_{SC} = 0.676$) was equally affected by juvenile rosette size, juvenile establishment time, and SLA; days to first flower, adult rosette size, and seed weight were heavily weighted on the two significant adult composite traits; and the physiological trait was almost entirely driven by N_{mass}. For both adult and juvenile multivariate analyses, Q_{SC} values for composite traits with local adaptation were higher than univariate Q_{SC} values for their component traits, supporting multivariate response to selection.

TRAIT COVARIANCE AND DIFFERENTIATION IN THE UNITED STATES VERSUS CHINA

We compared **G** matrix structure for each trait set in the United States and China to determine whether each country had similar underlying covariance structures. Similar structure indicates that traits covary similarly in invasive and native populations, and that genetic constraints imposed by trait associations would also be

Table 1. Variance components and Q_{CT} values for 13 phenotypic traits calculated for 10 invasive (China) and 10 native (United States) populations of *Geranium carolinianum*.

Trait	V_{reg}	V_{pop}	V_{fam}	V_{res}	Q_{CT}
Juvenile establishment time (days)	0.000	0.001	0.001	0.004	0.062
Juvenile rosette size (cm)	0.202	0.190	0.147	0.144	**0.294***
SLA ($mm^2\ mg^{-1}$)	0.000	0.013	0.013	0.193	0.000
Adult rosette size (cm)	0.000	4.994	7.334	27.509	0.000
N_{mass} (%)	0.097	0.050	0.000	0.212	**0.661****
$\delta^{13}C$ (%)	0.184	0.121	0.253	0.352	0.227
Height (cm)	7.059	25.430	53.482	32.720	0.051
Days to first flower	49.556	35.380	82.035	29.632	0.199
Reproductive ratio ($g\ g^{-1}$)	0.000	0.001	0.002	0.003	0.064
Aboveground biomass (g)	0.568	1.234	0.682	1.926	0.179
Ten-seed weight (g)	1.62×10^{-5}	2.35×10^{-5}	4.29×10^{-5}	8.07×10^{-6}	0.129
Neutral F_{CT}					0.067

V_{reg}, variance between regions (China and United States); V_{pop}, variance among populations within regions; V_{fam}, four times the variance among families within populations; V_{res}, residual variance.

Q_{CT} values marked in bold are significantly different from F_{CT}.

***$P < 0.025$, ****$P < 0.0001$.**

similar. If **G** differs in U.S. and Chinese populations, correlations may affect trait evolution differently in each country.

U.S. and China **G** matrices are unrelated for adult and juvenile trait sets in the CPCA (Table 6). We could not compare U.S. and China physiological matrices with CPCA because the analysis did not converge. When comparing the direction of the major axis of genetic variance for each trait set, we found moderate to high values of θ for all comparisons (Table 7), with the largest differences between countries being in adult traits ($\theta = 78.9$). Thus, **G** matrix structure differs between countries and may result in different genetic constraints to trait evolution in U.S. and Chinese populations.

To compare the composite traits under selection in each country, we additionally calculated θ for the primary eigenvectors of $\mathbf{F_{SCq}}$ for each set of traits. Similar eigenvectors in the United States and China (θ close to zero) would indicate that the direction of adaptive divergence was similar in the United States and China. We found θ values to be moderate (Table 7) for all sets of traits, indicating that adaptive trajectories differed between countries; that is, different adaptive trait combinations were favored in the United States and China.

CLIMATE

In the global climate PCA, Chinese and U.S. populations separated along the first and second PC axes. Climate PC1 explained 64.5% of the total variation. Chinese populations experience colder, drier winters and increased seasonality in both temperature and precipitation, characteristic of the continental climate of inland China. U.S. populations have relatively warmer, wetter winters with reduced seasonality, characteristic of the humid, warm temperate climate of the southeastern United States. *t*-tests confirmed that Chinese populations experience significantly colder winters, hotter summers, and increased seasonality than U.S. populations. The second global climate axis accounted for 31.7% of the total variation and was highly correlated with latitude ($P < 0.0001$, $r^2 = 0.813$).

Additional principal components analyses on the seven bioclimatic variables were performed separately for each country. Climate PC1 explained 64.0% of the climatic variation in China and 74.1% in the United States. Both U.S. climate PC1 and China climate PC1 were highly correlated with latitude (China: $P = 0.0002$, $r^2 = 0.83$; United States: $P = 1.0 \times 10^{-7}$, $r^2 = 0.97$). Although the sampled populations in the United States span a much wider range of latitudes than China (10° vs. 5°), in both countries southern populations tend to have warmer, wetter winters compared to northern populations.

Only traits with adaptive population differentiation within countries ($Q_{SC} > F_{SC}$) were compared to patterns in climate. Within China, these were juvenile rosette size, juvenile establishment time, SLA, aboveground biomass, N_{mass}, the first two juvenile composite traits, the first two adult composite traits, and the physiological composite trait. Within the United States, these were juvenile rosette size, N_{mass}, aboveground biomass, the first juvenile composite trait, the first two adult composite traits, and the physiological composite trait. Of these, four traits—one in China and three in the United States—were significantly associated with climatic variation (Fig. 2). In China, this was the physiological composite trait ($P = 0.043$, $r^2 = 0.348$). Northern Chinese populations, which experienced colder, drier

Table 2. Eigenanalysis of the matrices of multivariate regional differentiation (F_{CTq}) and tests for response to selection on composite traits ($Q_{CT} - F_{CT}$) for populations from the native and invasive ranges of *Geranium carolinianum*.

(a) Global Juvenile Traits

Trait	x_1	x_2
Eigenvalue (Q_{CT})	0.185	0.000
Percentage of variance explained	99.7	0.3
Cumulative percent variance	99.7	100
Trait loadings:		
Juvenile rosette size	0.944	0.198
Juvenile establishment time	−0.314	0.781
SLA	0.099	0.592

(b) Global Adult Traits

Trait	$\mathbf{x_1}$	$\mathbf{x_2}$	$\mathbf{x_3}$	$\mathbf{x_4}$	$\mathbf{x_5}$
Eigenvalue (Q_{CT})	**0.287***	0.040	0.019	0.016	0.011
Percentage of variance explained	76.4	10.6	5.1	4.2	3.0
Cumulative percent variance	76.4	87.0	92.0	96.2	99.2
Trait loadings:					
Days to first flower	0.436	−0.347	−0.555	−0.226	0.231
Adult rosette size	0.000	0.227	0.269	−0.933	−0.054
Height	0.525	−0.478	0.097	−0.126	−0.035
Ten-seed weight	−0.257	0.304	−0.755	−0.176	0.062
Aboveground biomass	−0.662	−0.711	−0.047	−0.176	−0.152
Reproductive ratio	−0.174	−0.054	0.194	−0.019	0.957

(c) Global Physiological Traits

Trait	$\mathbf{x_1}$
Eigenvalue (Q_{CT})	**0.485****
Percentage of variance explained	100
Cumulative percent variance	100
Trait loadings:	
N_{mass}	−0.997
$\delta^{13}C$	0.079

For $Q_{CT} - F_{CT}$ tests, *$P < 0.025$, ****$P < 0.0001$.

winters, tended to have higher N_{mass} and higher water-use efficiency. In the United States, juvenile rosette size was the only univariate trait significantly correlated with climate ($P = 0.013$, $r^2 = 0.506$). From the multivariate analysis, the first adult composite trait was significantly correlated with U.S. climate PC1 ($P = 0.0003$, $r^2 = 0.795$), as was the first juvenile composite trait ($P = 0.050$, $r^2 = 0.323$). Northern U.S. populations, which also experienced colder, drier winters, tended to have smaller, early establishing rosettes with thicker leaves, later flowering, and greater biomass at harvest.

Inferring agents of selection on multivariate traits must be approached with caution. Because multivariate adaptation is influenced by both selection on trait combinations and underlying genetic covariances, response to selection measured in $\mathbf{F_{SCq}}$ may not reliably reflect the direction of phenotypic selection (Chenoweth and Blows 2008). This may have affected correlations of composite traits with climate PC axes. However, multivariate trait correlations were consistent with univariate trait correlations, suggesting that adaptive composite traits reflect the direction of phenotypic selection.

Discussion

Invasive and native populations can diverge due to stochastic and selective processes (Keller and Taylor 2008). Additionally, patterns of adaptation among populations may differ between the invasive and native ranges as a result of altered selective pressures, changes in genetic variation, and/or changes in underlying trait correlations (the **G** matrix). Here, we found that phenotypic differences both between and within native and invasive ranges of *G. carolinianum* are being driven by drift, selection, and possibly altered trait correlations.

Table 3. Variance components and Q_{SC} values for 13 phenotypic traits calculated for 10 populations in each of the invasive (China) and native (United States) ranges of *Geranium carolinianum*.

Trait	V_{pop}	V_{fam}	V_{res}	Q_{SC}
(a) China				
Juvenile establishment time	0.001	0.001	0.005	**0.311******
Juvenile rosette size	0.048	0.095	0.110	**0.201****
SLA	0.021	0.000	0.177	**1.000******
Adult rosette size	0.814	2.622	27.125	0.134
N_{mass}	0.014	0.022	0.180	**0.251****
$\delta^{13}C$	0.051	0.125	0.225	0.169
Height	3.614	26.892	32.713	0.063
Days to first flower	3.201	25.284	30.360	0.060
Reproductive ratio	0.000	0.000	0.003	**0.000****
Aboveground biomass	0.266	0.352	1.481	**0.274*****
Ten-seed weight	3.74×10^{-6}	1.21×10^{-5}	6.65×10^{-6}	0.1333
Average Q_{SC}				0.236
Neutral F_{SC}				0.082
(b) United States				
Juvenile establishment time	0.000	0.001	0.004	0.090
Juvenile rosette size	0.332	0.206	0.170	**0.446****
SLA	0.005	0.036	0.195	0.069
Adult rosette size	9.299	12.167	26.834	0.276
N_{mass}	0.086	0.000	0.229	**1.000******
$\delta^{13}C$	0.191	0.394	0.454	0.195
Height	46.825	77.150	32.010	0.233
Days to first flower	68.016	139.288	26.367	0.196
Reproductive ratio	0.001	0.003	0.003	0.130
Aboveground biomass	2.177	1.041	2.182	**0.511*****
Ten-seed weight	4.36×10^{-5}	7.38×10^{-5}	9.10×10^{-6}	0.228
Average Q_{SC}				0.307
Neutral F_{SC}				0.196

V_{pop}, variance among populations; V_{fam}, four times the variance among families within populations; V_{res}, residual variance.
Q_{SC} values marked in bold are significantly different from F_{SC}.
$^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

Common garden measurements revealed genetically based differences between the U.S. and Chinese ranges in almost every trait. Such phenotypic differentiation between native and invasive populations has been demonstrated for many invasive species (e.g., Bossdorf et al. 2005; Felker-Quinn et al. 2013), but most studies do not distinguish between divergence due to selection and genetic drift. By comparing quantitative trait and neutral marker variation, we show that much of the phenotypic divergence between countries is indistinguishable from drift and cannot be attributed to selection. However, we did not have the statistical power to determine whether a lack of regional trait divergence (low Q_{CT}) was a result of unifying selection, because neutral divergence (F_{CT}) was low and because only two regions were used. Of the 11 traits in the univariate analysis, this lack of power would only possibly affect our results for SLA and adult rosette size, which had no regional differentiation ($Q_{CT} = 0$), but were not significant in the $Q_{CT} - F_{CT}$ test. All other traits had Q_{CT} values close to or much greater than F_{CT}.

Patterns revealed by the few traits that adaptively diverged between the two countries suggest that invasive populations are responding to inland China's continental climate, which has longer, harsher winters and stronger seasonal variation than the eastern United States. Relative to U.S. populations, Chinese populations have smaller rosette sizes and total aboveground biomass, with higher leaf N, later flowering, and taller inflorescences. As *G. carolinianum* is a spring-flowering, winter annual, some of these traits would be beneficial for colder drier winters in China, such as smaller rosettes to reduce water loss and later flowering to coincide with later springs. We also found significantly higher RMR in China, and although we were unable to test whether this was due to local adaptation, it is consistent with decreased growing season water availability. Additionally, the colder winters and hotter

Table 4. Eigenanalysis of the matrices of multivariate population differentiation (F_{SCq}) and tests for response to selection on composite traits ($Q_{SC} - F_{SC}$) for 10 populations from the invasive (China) range of *Geranium carolinianum*.

(a) China Juvenile Traits

Trait	x_1	x_2
Eigenvalue (Q_{SC})	**0.988******	**0.182***
Percentage of variance explained	84.3	15.5
Cumulative percent variance	84.3	99.8
Trait loadings:		
Juvenile rosette size	−0.133	0.690
Juvenile establishment time	0.389	0.697
SLA	0.911	−0.197

(b) China Adult Traits

Trait	x_1	x_2	x_3	x_4	x_5
Eigenvalue (Q_{SC})	**0.917******	**0.319******	0.093	0.039	**0.000***
Percentage of variance explained	67.0	23.3	6.7	2.8	0
Cumulative percent variance	67.0	90.3	97.1	100	100
Trait loadings:					
Days to first flower	0.303	0.096	0.663	0.490	−0.079
Adult rosette size	−0.608	−0.378	0.199	−0.275	−0.456
Height	−0.145	0.094	−0.423	0.665	−0.578
Ten-seed weight	−0.203	−0.469	0.448	0.266	0.094
Aboveground biomass	0.556	−0.760	−0.277	0.007	−0.088
Reproductive ratio	−0.408	−0.201	−0.254	0.414	0.660

(c) China Physiological Traits

Trait	x_1
Eigenvalue (Q_{SC})	**0.990******
Percentage of variance explained	93.2
Cumulative percent variance	93.2
Trait loadings:	
N_{mass}	−0.773
$\delta^{13}C$	0.634

For $Q_{SC} - F_{SC}$ tests, $^{*}P < 0.025$, $^{****}P < 0.0001$.

summers experienced by Chinese populations may also explain the smaller size of Chinese plants. With shorter fall and spring growing seasons, plants may not be able to accumulate as much biomass before flowering.

Although our results show that *G. carolinianum* in China has adapted to novel climatic conditions, there is little evidence that Chinese populations are evolving traits associated with increased invasiveness. Invasive traits are generally associated with high growth rates, reduced root allocation, and high fecundity (Pysek and Richardson 2007; van Kleunen et al. 2010). Some invasive species may evolve these traits after release from native antagonists allowing resources to be reallocated toward growth and competition, which is predicted by the EICA hypothesis. For *G. carolinianum*, however, increased leaf N was the only putatively invasive trait that we found to have adaptively increased in China. Although leaf N is positively correlated with photosynthetic capacity and is generally indicative of a faster growing, resource-acquisitive ecological strategy (Reich et al. 1994; Cornelissen et al. 1997; Wright et al. 2004), it has not resulted in faster growth in Chinese *G. carolinianum*. Despite higher leaf N, Chinese plants were smaller, suggesting slower, not faster, growth. A possible explanation for these contrasting traits is that selection for increased competitive ability (i.e., higher leaf N) could have been offset by selection imposed by the shorter growing season and/or increased abiotic stress caused by colder, drier Chinese winters. Overall, we found that selection during or after the establishment of *G. carolinianum* in China has been in response to the continental Chinese climate, rather than an increase in "invasive" traits due to release from native antagonists or other factors. This is consistent with its status as a minor invasive weed and suggests that, in the short term, *G. carolinianum* is unlikely to become a problematic invader.

Table 5. Eigenanalysis of the matrices of multivariate population differentiation (F_{SCq}) and tests for response to selection on composite traits ($Q_{SC} - F_{SC}$) for 10 populations from the native (United States) range of *Geranium carolinianum*.

(a) U.S. Juvenile Traits		
Trait	x_1	x_2
Eigenvalue (Q_{SC})	**0.676******	0.327
Percentage of variance explained	66.7	32.2
Cumulative percent variance	66.7	98.9
Trait loadings:		
Juvenile rosette size	−0.596	0.803
Juvenile establishment time	−0.599	−0.437
SLA	−0.534	−0.406

(b) U.S. Adult Traits					
Trait	x_1	x_2	x_3	x_4	x_5
Eigenvalue (Q_{SC})	**0.971******	**0.528******	0.249	0.112	0.069
Percentage of variance explained	50.4	27.4	12.9	5.8	3.6
Cumulative percent variance	50.4	77.8	90.7	96.5	100
Trait loadings:					
Days to first flower	0.715	−0.112	0.079	0.120	0.045
Adult rosette size	−0.516	0.520	0.238	0.328	0.002
Height	0.061	0.252	0.217	−0.416	0.844
Ten-seed weight	0.018	0.217	−0.903	0.230	0.282
Aboveground biomass	0.460	0.753	0.101	0.073	−0.262
Reproductive ratio	0.089	−0.196	0.253	0.804	0.372

(c) U.S. Physiological Traits	
Trait	x_1
Eigenvalue (Q_{SC})	**1.000******
Percentage of variance explained	84.7
Cumulative percent variance	84.7
Trait loadings:	
N_{mass}	−1.000
$\delta^{13}C$	0.040

For $Q_{SC} - F_{SC}$ tests, ****$P < 0.0001$.

Patterns of local adaptation among invasive populations will not necessarily mirror patterns in native populations for a variety of reasons. Even if selective pressures remain the same, which is unlikely, genetic variance for particular traits and trait combinations may change in invasive populations as a result of founder effects and affect the response to selection. In some cases, founder effects and genetic bottlenecks may actually increase genetic variance for quantitative traits via conversion of nonadditive genetic variance to additive genetic variance (Carson 1990; Neiman and Linksvayer 2005). Although we would not be able to detect such a conversion here because of our use of broad-sense heritabilities, a restructuring of **G** may make novel phenotypic combinations available and accelerate adaptive differentiation (Carson 1990).

We found a reduction in genetic variance for some, but not all, quantitative traits in Chinese populations when compared to U.S. populations, which parallels the reduction in neutral genetic diversity due to founder effects during colonization (Shirk et al. 2014). Despite this, more traits were under selection (as evidenced by $Q_{SC} \neq F_{SC}$) in Chinese than in U.S. populations, which is consistent with the rapid response of newly established Chinese populations to novel selection pressures. In the United States, populations were more highly divergent in neutral and quantitative traits, although fewer traits had a signature of selection. Higher mean Q_{SC} in the United States is due in part to the accumulated effects of drift over a longer evolutionary history in the region; however, these populations have also been exposed to selection for much longer than the Chinese populations.

Although adaptive differentiation between China and the United States was consistent with their climatic differences, for the most part climate is not driving divergence among populations within China. This is surprising, given that many studies have demonstrated the evolution of latitudinal clines in invasive populations for a number of traits, including flowering phenology,

Table 6. CPCA of relatedness in genetic covariance matrices for adult and juvenile traits, compared between United States and Chinese populations. The chi-square test (χ^2, df, *P*) compares each model against the next lower in the Flury hierarchy ("step-up approach"): equal, proportional, common principal components, and unrelated. AIC values are for the "higher" model and are used for the model-building approach, with the model with the lowest AIC value preferred.

(a) Adult Traits

Higher	Lower	χ^2	df	*P*	AIC
Equal	Proportional	548.855	1	0.0000	7335.649
Proportional	CPC	2660.606	5	0.0000	6788.795
CPC	CPC(4)	2114.043	1	0.0000	4138.189
CPC(4)	CPC(3)	766.512	2	0.0000	2026.146
CPC(3)	CPC(2)	240.672	3	0.0000	1263.634
CPC(2)	CPC(1)	663.994	4	0.0000	1028.962
CPC(1)	Unrelated	340.968	5	0.0000	372.968
Unrelated	—				42.000

(b) Juvenile Traits

Higher	Lower	χ^2	df	*P*	AIC
Equal	Proportional	209.182	1	0.0000	3562.896
Proportional	CPC	1934.383	2	0.0000	3355.714
CPC	CPC(1)	1130.214	1	0.0000	1425.331
CPC(1)	Unrelated	289.117	2	0.0000	297.117
Unrelated	—				12.000

Table 7. Similarity between the first eigenvectors of genetic covariance matrices (**G**) and the first eigenvectors of matrices of multivariate population differentiation ($\mathbf{F}_{SCq}$) in the United States and China. θ is the angle between the primary eigenvector of each matrix and ranges from zero (eigenvectors are equal) to 90 (eigenvectors are orthogonal).

Matrix	θ
G (juvenile traits)	60.49
G (adult traits)	78.91
G (physiological traits)	36.14
$\mathbf{F}_{SCq}$(juvenile traits)	50.15
$\mathbf{F}_{SCq}$ (adult traits)	42.51
$\mathbf{F}_{SCq}$ (physiological traits)	37.06

biomass, and physiology (Maron et al. 2007; Dlugosch and Parker 2008b; Montague et al. 2008; Colautti et al. 2009). Additionally, we found strong trait associations with latitude and climate in the United States, with northern populations possessing adaptations for longer, drier, and colder winters: smaller, earlier-establishing rosettes with thicker leaves and later flowering. In contrast, only the multivariate physiological trait (nearly equally weighted for leaf N and water-use efficiency) in Chinese populations was correlated with climatic and latitudinal variation. The adaptive value of this multivariate trait is less clear, but it may reflect adaptation to drier environments. Populations in colder, drier northern areas had increased leaf nitrogen and water-use efficiency, and it has been suggested that increased photosynthetic capacity (due to an increase in N-rich rubisco) allows a leaf to more efficiently use internal CO_2 when stomata are closed and gas exchange is limited (Wright et al. 2004).

Thus, it appears that climate is not the major factor driving adaptive evolution in China. However, it is also possible that our sampling limited our ability to detect a cline in some traits. In particular, the range of latitudes sampled in China (approximately 28°N–33°N) may not have been sufficient to generate a detectable trait–climate association. Another possibility is that younger populations on range edges remain in the lag period that is often necessary for recombination to generate locally adapted genotypes; in this case, any cline would not be easily detectable until they are fully adapted to local conditions.

By comparing the direction of multivariate adaptation, represented by the major eigenvectors of $\mathbf{F}_{SCq}$ in both countries, it is clear that selection is leading to different adaptive trait combinations in the two countries. And as discussed above, we found evidence that agents of selection likely differ between countries—climate/latitude gradients in the United States and unknown sources in China. However, because the $Q_{ST} - F_{ST}$ test measures adaptation (i.e., response to selection), differences found in $\mathbf{F}_{SCq}$ structure between countries may be due to differences in selection regimes, differences in trait associations (represented by **G**), or both. Furthermore, genetic correlations arising from linkage can be shaped by selection and genetic drift. **G** has been shown to vary across populations, often in response to selection (Cano et al. 2004; Eroukhmanoff and Svensson 2011), and we found large differences in average **G** of Chinese and U.S. populations for all three sets of traits. Although drivers of

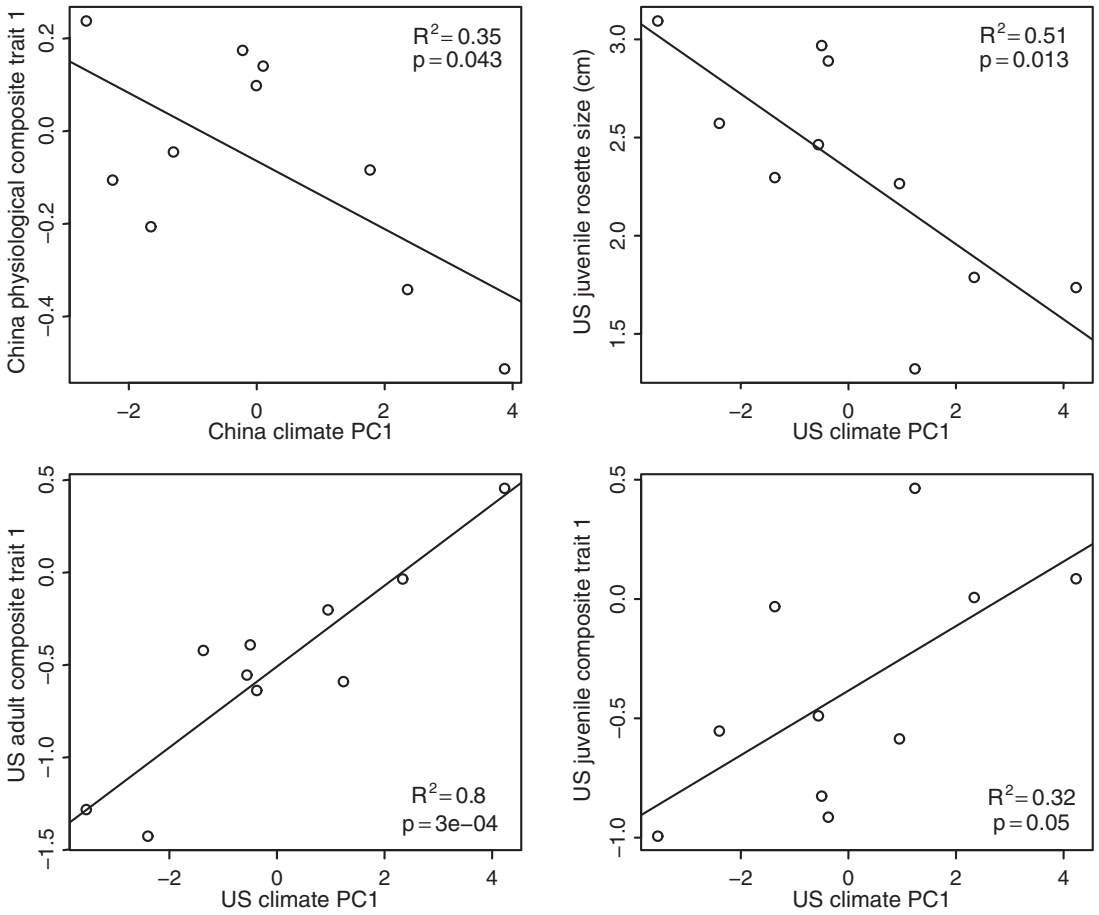

Figure 2. Significant correlations between climate and adaptive traits. For both China and U.S. climate PC1, more negative values indicate warmer, wetter winters.

differences between countries are unknown, it is possible that different selection regimes in China played a role in restructuring **G**. In invasive reed canary grass, *Phalaris arundinacea*, latitude (northern vs. southern populations) has a larger effect on the structure of **G** than provenance (invasive vs. native populations). These results suggest that for *P. arundinaca*, selection for latitudinal clines in both native and invasive ranges has had a larger effect on **G** structure than nonadaptive processes during colonization of the invasive range (Calsbeek et al. 2011).

Two univariate traits had the maximum Q_{SC} value of 1.00: SLA in China and N_{mass} in the United States. These traits had zero variance among families within populations (V_{fam}), and high residual variances—that is, we measured no heritable variation. Such a pattern is biologically possible, if these traits have experienced extremely strong selection that has exhausted additive genetic variance within individual populations. However, it is possible that for these traits, low levels of among-family variance may have been obscured by small measurement errors. Increasing sample size by including more families, with more individuals per family, may resolve this issue.

Multivariate Q_{SC} values were often, but not always, larger than univariate Q_{SC} values for the component traits, which indicate that selection generally was acting on trait combinations rather than single traits. However, one trait, juvenile rosette size, had a significant $Q_{SC} > F_{SC}$ in the univariate analysis, but not in the multivariate analysis. There are several possible reasons why a trait may have had a significant $Q_{SC} - F_{SC}$ pattern in one analysis but not the other. First, relationships among variance components may have changed when trait covariances were included in the multivariate analysis. Additionally, we were able to account for herbivory by including damage as a random covariate in the univariate but not the multivariate model. Finally, differences in estimation algorithms between two REML programs used for the univariate and multivariate modeling may have led to slightly different variance component estimates.

We found the signature of adaptation for many phenotypic traits in the U.S. and Chinese ranges of *G. carolinianum*, and multivariate analysis of adaptive trait differentiation revealed additional patterns of selection not observed in the univariate analysis. However, there is little evidence in China for the evolution of

traits associated with invasiveness. We found different adaptive trajectories in U.S. and Chinese populations, which are likely due to variation in selection regimes, but also possibly result from differences in trait covariances between regions. This suggests that the genetic effects of founding events and colonization may extend beyond a reduction in genetic diversity. Further work is needed to understand how these events change the structure of **G**, and how this affects the adaptive potential of colonizing populations.

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DATA ARCHIVING

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Supporting Information Methods for allozyme genotyping.