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Adaptive response in invasive ragweed

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Rapid evolution of invasive traits facilitates the invasion of common ragweed, Ambrosia artemisiifolia

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ABSTRACT

1. Invasive alien plants, together with organisms introduced for biological control, are ideal study systems with which to address questions of whether, and how fast, organisms adapt to changing environments. We compared populations of common ragweed, *Ambrosia artemisiifolia*, from native (USA) and introduced (China) ranges at similar latitudes, together with herbivores introduced for biological control, to understand the rate of evolutionary adaptive response of an invasive plant to novel environments.

2. Evolution of phenotypic traits associated with invasiveness was assessed by comparing differentiation in quantitative traits ($Q_{ST}$) to that of neutral microsatellite genetic loci ($F_{ST}$) and through climate data. A common-garden experiment estimated quantitative genetic variation associated with competition with grasses and biological control history by beetles.

3. Three growth traits (height, total, and stem biomass) and plasticity associated with additional nutrients were significantly greater in invasive compared to native populations and differed from expectations from genetic drift alone. Native, but not invasive, populations exhibited traits showing evidence of past selection and correlations with climate, consistent with the recent timing of introductions. Competition experiments between invasive populations and a US bunch grass showed reduced competitive ability in populations with a history of

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biological control, that might indicate a trade-off between competitive ability and herbivore resistance in invasive populations.

4. Our results demonstrate the rapid rate at which traits favouring invasion can evolve in invasive weeds, such as A. artemisiifolia, but also that adaptation may reflect joint effects of release from specialist herbivores and novel climatic conditions.

Key words: adaptive divergence, alien invasive species, biological control, China, past selection, $Q_{ST}-F_{ST}$, USA

INTRODUCTION

Biological invasions typically involve human-aided long-distance dispersal of species to regions where they have not previously occurred and which they cannot reach naturally. Invasions are generally characterized by significant demographic events including founder effects, population bottlenecks, multiple colonization events, hybridization, and range expansion, all of which influence the amount of genetic variation in invading populations and subsequent potential for adaptation to novel environments (Prentis et al., 2008, Bossdorf et al., 2005). For example, Shirk et al. (2014) showed that reduced diversity in invasive populations of Chinese Geranium carolinianum (Carolina cranesbill) has resulted from successive founder events during range expansion. On the other hand, genetic diversity can be higher in introduced as compared to native populations due to multiple introductions from genetically differentiated native populations (Genton et al., 2005, Kolbe et al., 2004). In addition to demographic events, species invasions are increasingly recognized as promising systems for studying adaptive evolution over contemporary time scales (Keller and Taylor, 2008). Indeed, much work suggests adaptive change of invasive species to be common in novel environments (Sakai et al., 2001, Prentis et al., 2008, Phillips et al., 2006).
Changes in the abiotic environment of the species' physiological niche can act as a force of selection during invasions. Because of the covariance between latitude and many aspects of the abiotic components (e.g., temperature, length of growing season, precipitation, soil characteristics), a response to physiological selection during the course of invasion may be evident in clines of quantitative traits with latitude across the introduced range (Broennimann et al., 2007). For instance, latitudinal clines in wing size have been observed among introduced Drosophila subobscura (fruit fly) (Gilchrist et al., 2004) and in several life history (e.g., flowering time, fecundity) and physiological traits (e.g., biomass, leaf carbon) of invasive plants, including Hypericum perforatum (St. John's Wort) (Maron et al., 2007) and Eschscholzia californica (California poppies) (Leger and Rice, 2007). Further, there is growing evidence that alien invaders often show divergence from their native populations in traits that increase their invasiveness (Colautti and Lau, 2015, Hodgins and Rieseberg, 2011). Blumenthal and Hufbauer (2007) found a consistent pattern of significantly larger plants in introduced than native plant populations across 14 different invasive species. Changes in the biotic environment, i.e. the release from specialized herbivores in the introduced range (Keane and Crawley, 2002), have also been proposed as prompting evolution in introduced populations. The Evolution of Increased Competitive Ability (EICA) hypothesis posits that selection in introduced populations favors genotypes with improved competitive ability at the expense of reduced herbivore defense, as defense against specialist herbivores no longer increases fitness (Blossey and Nötzold 1995). This trade-off can be examined by comparing native and introduced populations (Bossdorf et al., 2004) and/or plant populations with a long history of enemy exposure to those in which the enemy has only been observed for limited time or is still absent (Handley et al., 2008). Differences in phenotypic plasticities of invasive species between their native and introduced ranges are well studied and documented (Hahn et al., 2012, Chen et al., 2010, Bossdorf et al., 2005), yet less is known whether the plastic responses of traits is the result of adaptation to novel environments (van Kleunen et al., 2011). Understanding the relative importance of adaptive change in the success of invasive species, however, remains challenging for several reasons. First, manipulative experiments or paired before/after comparisons are difficult and often not possible (Osenberg et al., 1996). Second, it may be difficult to detect evolutionary change since it is often masked by strong ecological effects, such as...
escape from natural enemies (Torchin et al., 2003). These difficulties can be overcome by comparing native populations to the introduced populations of an alien invasive species through experimental manipulations under controlled conditions in a ‘common garden’.

Divergence in neutral markers can be used as a baseline measure of a null expectation for the degree of population differentiation without selection, estimated by \( F_{ST} \) (Wright, 1951), which can then be compared with divergence in quantitative traits of interest (Leinonen et al., 2013, DeWoody et al., 2010, Leinonen et al., 2008, Steinger et al., 2008, Whitlock, 2008), estimated by \( Q_{ST} \) (Spitze, 1993). Recent studies document that adaptive evolution can occur over short timescales, and biologists have begun to examine adaptive evolution during biological invasions (Colautti and Lau, 2015, Xu et al., 2010, Müller-Schärer et al., 2004). Such evolutionary changes can occur not only between the native and introduced regions but also within the introduced region over time (Gruntman et al., 2017, Phillips et al., 2006), such as for organisms introduced for biological control (Roderick et al., 2012). Importantly, \( Q_{ST} \) is often ideally measured in a randomized ‘common garden’ experimental design to exclude effects on the trait associated with environmental differences between populations (with an appropriate design to partition the within-population variance, and preferably also to remove non-additive effects, such as dominance and epistatic effects).

Here, we study the notorious invasive ragweed, *Ambrosia artemisiifolia*, as a ‘natural’ experiment (Sax et al., 2007) to measure the relative importance of rapid evolutionary changes in the context of interacting biotic and abiotic conditions in the new environments as compared to the native region (Callaway and Maron, 2006, Roderick et al., 2012). By using a joint approach of population genetics and two common garden experiments, we address the following questions: i) are phenotypic differences between native and introduced regions the result of past selection; and if so, have invasive populations evolved traits associated with increased invasiveness? ii) Is climate a possible driver of evolution between two regions? iii) Do introduced populations show increased phenotypic plasticity for relevant traits, such as in response to nutrient availability? We also assess a) the role of selection in phenotypic differences within the invasive region in association with biological control history, and b) potential trade-offs between competitive ability and herbivore resistance in invasive populations.

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MATERIALS AND METHODS

Study species

Common ragweed, *Ambrosia artemisiifolia* L. (Asterales: Asteraceae), is a widespread annual weed native to North America and accidentally introduced to many parts of the world, including China, Russia, Europe, and Australia (Essl et al., 2015). In China, *A. artemisiifolia* was first recorded in Hangzhou, Zhejiang Province in 1935 (Wan et al., 1993), and has by now expanded to 21 provinces in northern, central, eastern, and southern China (Zhou et al., 2010). It was listed as a quarantined agricultural noxious weed in China in 1997 due to its particularly large production of highly allergenic pollen, its threat to agricultural systems, and its impact on native species and communities (Xie et al., 2001).

Study populations of *Ambrosia artemisiifolia*

Several insect herbivores of *A. artemisiifolia* were accidentally or deliberately introduced for potential biological control in China around 1990 (Gerber et al., 2011). After extended host specificity tests, mass-rearing programs releases of two herbivores, the leaf beetle *Ophraella communa* LeSage (Coleoptera: Chrysomelidae) and the tip-galling moth *Epiblema strenuana* Walker (Lepidoptera: Tortricidae) were initiated 10 and 25 years ago, respectively. Together with their natural spread, selected mass releases have resulted in a mosaic of *A. artemisiifolia* populations with respect to specialist herbivore impact: some populations have experienced very strong top-down regulation, while other populations have not yet experienced any herbivores released for biological control herbivores and remain free of herbivory (SS Zhou, personal communication).

In October 2013, seeds from 10 Chinese (CN) populations were collected from sites at least 20 km apart with and without biological control history based on available records and personal observations (Fig. 1, Table S1). For each population, seeds were obtained from a minimum of 15 randomly chosen mother plants. Seeds within maternal families were considered half-sibs, given that

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A. artemisiifolia is predominantly outcrossing, with very high outcrossing rates (0.93–1.0) for both Chinese and North American populations (Friedman and Barrett, 2008, Li et al., 2012). In addition, a bulk sample of seeds was collected from each population from a minimum of 100 randomly selected plants. We also obtained seeds from 10 populations from the native United States (US), with 7-15 mother plants per population (Fig. 1, Table S1). Populations from both ranges were collected in non-agricultural, mainly ruderal/disturbed or semi-natural sites. In April 2014, seeds were transferred to moist filter paper in Petri dishes with a cold-stratification period (4°C and 24-h darkness) for 4 weeks to break primary dormancy (Willemsen, 1975).

Analysis of neutral genetic diversity

In May 2014, 5-10 stratified seeds from all half-sib families were placed into Petri dishes containing two filter papers wetted with distilled water in a growth chamber for germination, following a 12-h day regime with 20°C day and 10°C night temperature to simulate early spring conditions (Leiblein-Wild et al., 2013). If necessary, distilled water was added to ensure sufficient moisture. Germinated seeds were put into seedling trays with 150 (10 × 15) cell plugs of 15 ml volume filled with commercial potting soil for initial growth for two weeks. The seedling trays were placed on tables in a glasshouse (Oxford Tract, UC Berkeley). Seedlings were transplanted into 1 L plastic pots, filled with a mixed substrate of 50% vermiculite and 50% commercial potting soil and grew for 1.5 months. In total, we obtained fresh leaf samples from 273 young A. artemisiifolia individuals (176 from China, 97 from the US). Genomic DNA was extracted using Qiagen DNeasy™ plant Mini kit and diluted 10 times. We used the following 21 microsatellite (simple sequence repeat, SSR) markers: Amb 12 and 82, GenBank accession no: AY849308, AY849312, Genton et al. (2005); Ambart 04, 06, 09, 17, 18, 21, 24 and 27, GenBank accession no: FJ595149 – FJ595156, Chun et al. (2010); ambel454-SSR 10, 17, 26, 39, 47, 54, 67, 71, 73, 86 and 91, GenBank accession no: KX867681, KX867684, KX867687, KX867691, KX867694, KX867695, KX867699, KX867702, KX867703, KX867706, KX867707, Meyer et al. (2017). All forward primers were modified to a fusion of leading M13(-21) 18bp

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universal sequence (5’-TGT AAA ACG ACG GCC AGT-3’) with the original primer sequence (Schuelke, 2000). All primers and a FAM-labeled M13(-21) fluorescent universal primer were supplied by Sigma-Aldrich (United States). The PCR mix contained ~10 ng genomic DNA, 0.04 μM forward primer, 0.16 μM reverse primer, 0.16 μM FAM-M13(-21) primer, 0.5 U Taq DNA polymerase (Qiagen), in a final 10 μl reaction volume (PCR buffer including 67 mM pH 8.8 Tris-HCl, 16.6 mM (NH₄)₂SO₄, 2 mM MgCl₂, 0.7 mM β-mercaptoethanol, 0.7 mM each dNTP, 0.05% Brij® 58 (Sigma-Aldrich) and 0.2 mg/ml BSA). Conditions of the PCR amplification are as follows: 95˚C (15 min), then 30 cycles at 95˚C (30 s) / 50˚C (45 s) / 72 ˚C (45 s), followed by 8 cycles 95˚C (30 s) / 53˚C (45 s) / 72 ˚C (45 s), and a final extension at 72˚C for 5 min (Chun et al., 2011). Amplified products were genotyped using an ABI 3730XL DNA analyzer (Applied Biosystems™) with an internal 600 LIZ due size standards (Applied Biosystems) at UC Berkeley, DNA Sequencing Facility. Fragments were called and analysed using GeneMarker Software (Softgenetics, State College, PA).

Descriptive statistics for microsatellites including observed heterozygosity (Hₒ), expected heterozygosity (Hₑ), observed gene diversities (Hₗ), overall gene diversity (Hₜ), and inbreeding coefficients (Fᵢₛ) were estimated using the R package adegenet (Jombart, 2008) based on the allele designation from fragment size determinations and diveRsity with respective 5000 bootstrapped iterations to execute 95% confidence intervals (Keenan et al., 2013). Multilocus variance components and F-statistics were calculated using the package hierfstat in R (Goudet, 2005). Fₜ over regions and populations within regions were estimated by permutation tests, with 95% confidence intervals by the range of the central 95% of 5000 bootstrap estimates.

Common garden and phenotypic traits measurements

We conducted a common garden experiment to assess quantitative traits and phenotypic plasticity of native and invasive populations (Richards et al., 2006). We measured plasticity to nutrient addition as ragweed, besides being widely distributed in non-agricultural sites is also known as an important crop
weed both in native and in introduced ranges. The species is therefore expected to be exposed to both high and low soil nutrient conditions. In May 2014, 10-40 stratified seeds from 261 half-sib families were selected for germination and initial growth in seedling trays (see above for details). One seedling of each half-sib family was used for the microsatellite analysis and six more seedlings from the same family were used for a common garden experiment. Families with fewer than seven available seedlings were only used for microsatellite analysis, thus 12 families of 273 were not included in the half-sib design. After two weeks of growth, seedlings were transplanted into 1 L plastic pots, filled with a mixed substrate of 50% vermiculite and 50% commercial potting soil. Three seedlings per half-sib family received a fertilizer treatment (40 g of slow-release 14:7:24 Multicote fertilizer) and three seedlings served as controls. In total, there were 846 pots with 2 nutrient treatments × 3 replicates × (74 CN families from 10 CN populations + 67 US families from 10 US populations). All pots were exposed to natural light, supplemented by metal halide bulbs (18-h light, 6-h dark), at a temperature of 15–22°C. Plants were watered with tap water every second or third day.

All plants were harvested at the end of August 2014 and separated into vegetative and reproductive (racemes and seeds) parts, dried for 72h to a constant weight at 80°C and weighed to an accuracy of ±0.001g. We recorded traits related to growth (height, total aboveground biomass, stem biomass), reproduction (flower biomass, flower density, i.e. average flower number per cm shoot, number of flowering shoots), and phenology (seed status, i.e. whether any seeds were found when harvested). Phenotypic Plasticity Index (PI) of six traits was assessed by calculating $PI = \frac{F_N - F_C}{F_N}$, where $F_N$ is the trait value of each individual plant with additional nutrient treatment, and $F_C$ is the average trait value of the three individual plants from the same family under control treatment; the traits include: height, total biomass, stem biomass, flower biomass, flower density and number of flower shoots.
Differentiation in quantitative traits

Differences between regions for the above listed phenotypic traits and phenotypic plasticity indices were assessed using linear mixed-effects models and generalized linear mixed-effects models (LMM/GLMM) and fit using the *lmer/glmer* function obtained from the R package *lme4* that uses maximum likelihood to estimate the model parameters (Pinheiro et al., 2012). In the models, the region was included as a fixed effect, seed family was nested within populations, which in turn were nested within regions as random effects. The nutrient treatment was also included as a fixed effect for all seven phenotypic traits. Normality of the residuals of all models was assessed using QQ-plots and data were logarithm transformed (base 10) when necessary to meet the assumption that the model residuals are approximately normally distributed (log(y+0.1) and log(y+0.01) were also used for reproductive traits due to few non-flowering individuals).

We estimated narrow-sense heritability \( h_N^2 = \frac{V_A}{V_A + V_E} \) for all seven phenotypic traits and six PIs. The within-family variance, \( \sigma_F^2 \), estimated the environmental variance, \( V_E \), and the genetic variance due to residual additive genetic factors, \( \sigma_G^2 \), provided an estimate of the additive genetic variance, \( V_A \). The significance of heritability was tested by comparing the likelihood of the model in which \( \sigma_G^2 \) is constrained to zero with that of a model in which \( \sigma_G^2 \) is estimated. Details of all traits and PIs for which heritabilities were calculated are given in Table S2.

Quantitative trait differentiation between regions (\( Q_{CT} \)), among populations within each region (US \( Q_{SC} \) and CN \( Q_{SC} \)) and between populations differing in biological control history within CN (\( Q_{SC-h} \)) were calculated as \( Q_{CT} = \frac{\sigma_R^2}{\sigma_R^2 + \sigma_F^2 + 2\sigma_P}, \) \( Q_{SC} = \frac{\sigma_F^2}{\sigma_F^2 + 2\sigma_P} \) and \( Q_{SC-h} = \frac{\sigma_R^2}{\sigma_R^2 + \sigma_F^2 + 2\sigma_P} \), respectively, where \( \sigma_R^2 \) is the additive genetic variance between-region, \( \sigma_P^2 \) is the additive genetic variance among-population, \( \sigma_G^2 \) is the additive genetic variance between different biological control history within CN and \( \sigma_F^2 \) is the variance among families within populations, estimated as four times the observed variance among families to account for half-sib families (Lynch and Walsh, 1998). Although we assumed that the sampled families of *A. artemisiifolia* were composed of half-sibs due to very high outcrossing rates.
(see above), it is still possible that families included some full-sibs due to the fact that we collected seeds in the field. Our estimation of $\sigma^2_e$, therefore, may be inflated, biasing our $Q_{CT}$, $Q_{SC}$ and $Q_{SC:h}$ estimates downward (Lynch and Walsh, 1998), therefore, becoming conservative with respect to the past diversifying selection. Variance components were calculated using restricted maximum likelihood (REML) in LMMs/GLMMs with confidence intervals estimated from 5000 bootstraps over families. To obtain the variance components to calculate $Q_{CT}$, the model included family nested within the population which in turn was nested within region as random effects. For $Q_{SC}$, the model included family nested within the population as random effects. For $Q_{SC:h}$, the model included family nested within the population which in turn was nested within biological control history group as random effects.

Climate

Nine bioclimatic variables known from previous work to be important determinants of the suitable habitats worldwide of A. artemisiifolia (Sun et al., 2017) were extracted from WorldClim (Hijmans et al., 2005) to describe the climate in each population. These included mean annual temperature (bio1), isothermality (bio3), temperature seasonality (bio4), max temperature of warmest month (bio5), min temperature of coldest month (bio6), temperature annual range (bio7), mean temperature of driest quarter (bio9), precipitation of driest month (bio14) and the growth degree days (GDD > 8°) for A. artemisiifolia following the baselines of sunflower. The calculation of GDD was based on monthly average temperatures using Thom's formula (Thom, 1966). A principal components analysis (PCA) for all populations was calibrated to explore major climatic differences between the two regions. Two PCAs were performed separately for both regions to generate a composite climate axis for both regions (US climate PC1 and CN climate PC1). The phenotypic traits that carry the signature of adaptive differentiation between two regions ($Q_{CT} > F_{CT}$) were then plotted with their respective climate axes. Correlations were then examined using a linear regression model.

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Competition experiment

We conducted a series of pairwise competition experiments in the glasshouse (Oxford Tract, UC Berkeley) involving each of the 10 CN populations and a seedling of Koeleria macrantha, prairie junegrass, a bunch grass widespread across North America and Eurasia (including China) in a large number of habitat types, and widely co-occurring with A. artemisiifolia. In July 2014, we transplanted seedlings of A. artemisiifolia from bulk samples of each of 10 populations collected in CN into 1.5 L pots, competing with/without one seedling of K. macrantha, at a distance of 10 cm among seedlings in the competition pots. As a control, single K. macrantha plants were also transplanted into 10 pots without competitors. In total, we had 210 pots, i.e., 10 populations × 2 competition treatment × 10 replicates, as well as K. macrantha grown alone. After transplanting, plants experienced natural light conditions supplemented by metal halide bulbs (18-h light, 6-h dark), to a temperature of 15-22°C. Plants were manually watered with 100 ml tap water per pot every three days to ensure that each pot was given an equal amount of water. In order to reduce position effects, pots were re-randomized every two weeks.

Previous studies showed that individual plants of common ragweed reach their maximum height at flowering in a temporally consistent way (Sun and Frelich, 2011). Thus, all plants were harvested after 100 days, when 95% of the plants reached the flowering stage. Plants were separated into parts associated with growth and reproduction, dried for 72 h at 80°C to a consistent weight and weighed to an accuracy of ±0.001g. Reduction in biomass of K. macrantha was assessed by calculating: \( \frac{B^a - B^+}{B^a} \), where \( B^a \) is the average biomass of ten grass individuals grown in control pots in absence, and \( B^+ \) is the biomass of the grass grown in the presence of A. artemisiifolia in the competition pots.

LMMs were used to assess the effect of biological control history and climate condition of each population on the reduction in biomass of K. macrantha. In those models, biological control history, CN climate PC1 and their interactions (see above for details) was included as a fixed effect, and the population was treated as a random effect. Mixed-effect regression models fit using the lme function obtained from the R package nlme were used to analyse the relationship between the biomass of A.
artemisiifolia and that of K. macrantha in the competition pots. For this, we compared a random intercept and slope model and a random intercept model using population factor and we used the likelihood ratio test from restricted maximum likelihood fits for significance. As the results indicated no difference between the common slope and the slopes of each of the populations (P > 0.1), we thus examined the correlation in a generalized linear model. Due to the dependence of the biomass of the two species, we used model-II simple linear regression (geometric mean regression) fit using the lmodel2 function with standard major axis (MA) method obtained from the R package lmodel2 to estimate the relationship between the biomass of A. artemisiifolia and that of K. macrantha in the competition conditions. All biomass data were ln-transformed. All analyses and figures were performed using R statistical software, version R-3.3.2 (R Development Core Team, 2016).

RESULTS

Population genetic structure based on microsatellite markers in the native and introduced region

Of the 21 microsatellite loci tested, 14 were polymorphic across both the US and CN. The number of alleles per locus was 27.72 ± 2.47 (US) and 30.36 ± 2.27 (CN). Levels of within-population diversity estimated as expected heterozygosity ranged from 0.60–0.96 (US) and 0.56–0.95 (CN). All CN populations had FIS values significantly larger than zero, indicating an overall excess of homozygosity, while only half of the US populations had FIS values significantly greater than zero. All populations conformed to HW equilibrium after correcting for null alleles. Neutral marker analyses revealed little regional genetic differentiation between the US and CN (FCT = 0.004, 95% CI: 0.001–0.007), and within regions, among-population differentiation was similar in the invasive (CN FSC = 0.031, 95% CI: 0.019–0.043) and native region (US FSC = 0.026, 95% CI: 0.012–0.042). Within-population diversity was moderate with He = 0.69–0.80 in the US and He = 0.63–0.80 in CN.
Inheritance and differentiation in quantitative traits in the native and introduced regions

Estimates of heritability ($h^2$) ranged widely from 0 to 0.99 (Table S2). Heritability estimates of the seven phenotypic traits for the ten native US populations resulted in five times more significant $h^2$ values than for the invasive CN populations (20 vs. 4), while numbers of significant values for the six phenotypic plasticity indices were similar for the populations from the two ranges (19 vs. 21; significant $h^2$ in bold in Table S2). Average heritability in quantitative traits did not differ between the US and CN ($\chi^2 = 2.92, P = 0.09$; Table S2), but the heritable variation in phenotypic plasticity indices was significantly higher in CN than that in the US ($\chi^2 = 5.22, P = 0.02$; Table S2). Differentiations in four out of seven quantitative traits were significantly greater among US populations than among CN populations ($Q_{SC-US} > Q_{SC-China}$, Fig. 2), while the other quantitative traits did not differ between the two regions.

Genetic differentiation of neutral markers and quantitative traits

Between regions

LMMs showed significant differences between the US and CN for all three growth traits (height, total biomass, and stem biomass, $\chi^2 \geq 4.21, P \leq 0.04$; Table S3), all being larger in the CN populations, but no differences were found for the four reproduction traits ($\chi^2 \leq 2.08, P \geq 0.15$; Fig. S1, Table S3). All growth traits showed evidence of past selection ($Q_{CT} > F_{CT}$; Fig. 3, Table S4). Of the six phenotypic plasticity indices, values of two growth traits were significantly larger in the invasive CN than in the native US regions ($\chi^2 \geq 4.32, P \leq 0.04$; Fig. S1, Table S3). More specifically, the CN populations showed a greater performance (larger total biomass and stem biomass) response to nutrient addition than the US populations (Fig. S1, Table S3), but only plasticity in total biomass showed evidence of past selection ($Q_{CT} > F_{CT}$; Fig. 3, Table S4).

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Within regions

Within the US, all seven traits differed significantly among populations ($\chi^2 \geq 16.7, \ P < 0.05$; Table S5), and phenotypic plasticity indices of height and flower density varied significantly across populations ($\chi^2 \geq 17.03, \ P \leq 0.04$; Table S5). Two growth traits and three reproduction traits showed a significant $Q_{SC} > F_{SC}$, and $Q_{SC}$ values of all phenotypic plasticity indices were similar to $F_{SC}$ (Fig. 2a, Table S7).

Within CN, only height differed significantly among populations ($\chi^2 = 18.03, \ P = 0.03$, Table S6). Seed status and phenotypic plasticity index of flower density differed slightly ($\chi^2 = 15.83, \ P = 0.07$ and $\chi^2 = 16.34, \ P = 0.06$; respectively, Table S6), while all other phenotypic traits and plasticity indices did not differ among populations ($\chi^2 \leq 13.18, \ P \geq 0.16$; Table S6). Average $Q_{SC}$ across phenotypic traits in the US (0.280, average 95% CI: 0.12–0.40) was much higher than the average $Q_{SC}$ (0.008, average 95% CI: -0.06–0.07) in CN; similarly, for phenotypic plasticity index, values were 0.019 (average 95% CI: -0.03–0.066) vs. 0.004 (average 95% CI: -0.03–0.03) for US and CN, respectively. None of the traits of CN populations had greater $Q_{SC}$ to $F_{SC}$, and $Q_{SC}$ values for plasticity index of height and flower biomass were smaller than $F_{SC}$ (Fig. 2b, Table S7).

**Correlations between population trait means & climate conditions of their origin**

Climate PC1 explained 70% and PC2 21.4% of total climate variation across sample sites in CN and US. The sampled US populations experience cooler summers and increased seasonality, while CN populations have relative warmer summers and reduced seasonality, the typical characteristic of the temperate climate of eastern China. The first climate axis (PC1) was highly correlated with latitude ($r^2 = 0.83, \ P < 0.001$; Fig. S2). To compare the effects of respective climates on phenotypic differentiation, we mainly looked at traits with adaptive population differentiation between regions ($Q_{CT} > F_{CT}$). US climate PC1 explained 81% of the total climatic variation and was highly correlated with all four traits. Given that the climate PC1 is strongly correlated with latitude (Fig. S2), southern US populations that experienced warmer climate tended to grow taller and bigger and had less
reproductive allocation than northern US populations. For the CN populations, climate PC1 explained 88% of the total climatic variation, but with no significant correlation with any growth traits (Fig. 4). However, when considering only CN populations with biological control history, total biomass becomes highly correlated with climate PC1 ($r^2 = 0.5$, $P = 0.03$; Fig. S3).

**Effect of biological control history in the introduced CN region**

**Trait differentiations**

Total biomass showed evidence of being under selection ($Q_{SC-h} > F_{SC-h}$, Fig. 5) and slightly differed between populations with and without biological control history ($\chi^2 = 2.9$, $P = 0.09$, Table S8), with the latter ones being slightly larger. This is the reason why including the populations without biological control history eliminated the climate-plant size relationship (cf. above). Plasticity index of flower shoot numbers differed significantly between these two groups ($\chi^2 \geq 3.86$, $P < 0.05$; Fig. S4, Table S8), being larger in populations without biological control history. No significant results were found for all other traits ($\chi^2 \leq 0.74$, $P \geq 0.39$, Table S8).

**Competition experiment**

The reduction in biomass of *K. macrantha* was significantly greater when grown together with individuals of *A. artemisiifolia* populations that have no biological control history, compared to when grown with those with biological control history ($\chi^2 = 4.85$, $P = 0.03$, Fig. 6a), and was also significantly affected by CN climate PC1 ($\chi^2 = 3.91$, $P = 0.05$) and the interactions of biological control history and CN climate PC1 ($\chi^2 = 4.04$, $P = 0.04$). The biomass of *K. macrantha* decreased with increasing latitude for populations with biological control history, but not when populations were included with no biological control history (Fig. S5). When grown together, the biomass of *A. artemisiifolia* explained a significant part of the variation in biomass of *K. macrantha*, irrespective of biological control history ($R^2 = 0.20$, $\Delta\text{log-likelihood} = -72.67$, $P = 0.006$; Fig. 6b).

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DISCUSSION

Biological invasions are ‘natural’ experiments that can improve our understanding of contemporary evolution. Here, we provide evidence of differentiation and adaptive evolution in populations of the plant invader, *A. artemisiifolia*, at two nested spatial scales: (i) between native and introduced regions and (ii) between two groups of populations within the introduced region that either had or had not experienced biological control management though specialized herbivores.

**Differentiation between and within regions**

Using neutral molecular markers, our results reveal a similar genetic diversity between the introduced CN and native US region. This is consistent with patterns of genetic variation found previously across populations from 8 US states, and 19 European and 5 non-European (including China) regions (Gaudeul et al., 2011). Together with the observed moderate within-population diversity in both regions, and increased within-population inbreeding coefficient $F_{IS}$ in CN compared to the US, our results are consistent with less frequent introduction events into China and from a lower number of differentiated source populations and/or individuals than in introduced European populations (Gaudeul et al., 2011). All within-populations $F_{IS}$ values in the introduced CN region were significant, suggesting an overall excess of homozygosity that could be caused by crosses among neighbours that are genetically more closely related.

The results of our common garden experiment show that the amount of heritable trait variation varied among populations and regions. The higher number of significant heritability estimates of the phenotypic traits in the US as compared to CN populations suggests that genetics explains a greater portion of the phenotypic variation in the native *A. artemisiifolia* in the controlled environment in which it was measured. However, no such regional differences were found for the phenotypic plasticity indices. Furthermore, the increased phenotypic plasticity indices to nutrients of the CN populations as compared to the US populations indicates the potential of evolved plasticity to nutrients in the CN populations or alternatively the loss of plasticity in the native US populations. In

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fact, the lower heritability for plasticity of the US than the CN populations may indicate a potential constraint on the maximal amount of plasticity in response to nutrient addition of the US populations that can evolve, since low heritability values often reflect the impact of past selection that removed genetic variation for plasticity (Price and Schluter, 1991). If we assume the controlled experimental environments are reflective of nature, these results could be explained by the hypothesis that native US populations have reached their ecological niche boundary, while the CN populations are still expanding with plasticity being crucial for conquering a mosaic of soil nutrient conditions.

Overall, we find population differentiation to be lower in the introduced CN region. The reduced quantitative trait differentiation may be related to the more recent establishment, the evolution of phenotypic plasticity, or the frequently experienced genetic bottlenecks of the introduced populations, or a combination of these (Dlugosch and Parker, 2008). In line with previous studies documenting genetically based differences in multiple traits after introduction into new ranges (Felker - Quinn et al., 2013), our common garden measurements reveal smaller plants (height and biomass) and lower plasticity of growth traits in the native US as compared to the introduced CN region (Fig. S1), which might be due to biological trade-offs and different evolutionary trajectories in the two regions (cf. below). Although the distribution of ragweed in North America has experienced an expansion in abundance after European settlements (Bernabo and Webb, 1977, Lavoie et al., 2007), the spread in China is much more recent as it has only been reported since 1930s and has since extended its range from northern to southern China (Wan et al., 2005).

Testing for past selection

Between native and introduced regions

By comparing divergence in quantitative traits with neutral divergence inferred from microsatellite markers, our results indicate that three growth traits (height, total biomass and stem biomass) and the phenotypic plasticity index of total biomass had greater $Q_{CT}$ than $F_{CT}$, show past selective divergence.

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between regions. Phenotypic divergence of all other traits is indistinguishable from genetic drift between the two regions. The observed selection towards increased growth traits in the introduced region can be considered as adaptive as biomass was found in a previous study to be highly positively correlated with reproduction (seed and pollen) in *A. artemisiifolia*, assessed on 45 sites across various habitat types in Europe (Lommen et al., 2017). Larger size and higher plasticity of growth traits can be assumed to favour invasiveness. We did not observe any differences between the two regions in our measurements of reproduction, i.e. flower biomass, flower density and number of flower stems, which might be due to the fact that those traits may not be good proxies for the amount of seeds and pollen. More studies are clearly needed to elucidate these relationships.

Our results further show an increased response to nutrient addition in the introduced CN populations compared to native US populations, which is consistent with previous findings for other invasive species (Lafuma et al., 2003, Bossdorf et al., 2005). The introduced CN populations exhibited a ‘Jack-and-master’ strategy in response to additional nutrients (Richards et al., 2006), with a pronounced (plastic) response to exploit favourable environments (nutrient rich) indicated by an increased biomass as compared to the native US populations when fertilized, but less so in controls. *Ambrosia artemisiifolia* is reported to have been a severe agriculture weed in China for 30 years (Wan et al., 1993), which could be at least partly explained by evolution of high plasticity associated with different nutrient environments, which in turn may help drive its fast spread and invasion success.

Traits found to be under selection in the US populations (*Q*$_{SC-US}$ > *F*$_{SC-US}$) were highly correlated with climate variables, indicating their response the climate ranging from temperate in the southeast to continental climate in northeast of the US. Only two plasticity indices were found to be under uniform selection in the CN populations (*Q*$_{SC-China}$ < *F*$_{SC-China}$). However, the effects of nonadditive genetic variation often lead to underestimation of *Q*$_{ST}$ due to a large amount of dominance and epistatic genetic variance and make it difficult to draw inferences from *Q*$_{ST}$ < *F*$_{ST}$ that we observed. The fact that we found no genetic differentiation among the CN populations, no observations of *Q*$_{SC-China}$ > *F*$_{SC-China}$ for all traits, may be due to the time lag after their introduction some 80 years ago (Wan et al., 1993). The traits found to be under selection during or after the establishment of *A.*
artemisiifolia in the introduced CN region ($Q_{CT} > F_{CT}$) can be seen as a general response to increased invasiveness and spread across heterogeneous habitats, rather than to the local Chinese climate. This is consistent with A. artemisiifolia’s status as a major problematic invasive weed that spread rapidly and presently covers 21 provinces in China (Ma et al., 2008). We acknowledge that due to the high gene diversity found in our populations, the genetic differentiation ($F_{ST}$) is expected to be negatively biased downward as the mutation rate is likely high relative to the migration rate favouring the observation of $Q_{SC} > F_{SC}$ (Jost, 2008). Nevertheless, our results indicate a clear difference between the CN region ($Q_{SC-China} vs. F_{SC-China}$) as compared to the US region ($Q_{SC-US} > F_{SC-US}$).

Between sites differing in biological control history within China

Besides comparing native and introduced regions, we also explored potential adaptive evolution in invasive A. artemisiifolia within the introduced region due to exotic natural enemies that were initially accidentally or deliberately introduced. The two presently most widespread insect species were first reported in 1990 and soon thereafter mass released resulting in a biological control management of now more than 20 years. Because some populations have not yet experienced any biological control and have remained mainly free of any herbivory we could assess the importance of top-down regulation by biological control agents. Between these two groups, only total biomass differed: this trait was found to be under selection ($Q_{SC-h} > F_{SC-h}$) and to be higher in populations without biological control history. Larger plants were also found to be better competitors for resources as indicated by the significant negative relationship between the biomass of A. artemisiifolia and that of K. macrantha (Sun et al., 2013). Well in line with this, we found a significantly increased competitive ability of CN populations without a biological control history in the pair-wise competition experiment with K. macrantha (Fig. 6a). The fact that A. artemisiifolia plants were found to be generally larger in CN as compared to the native US populations could be explained by enemy release (or escape), as shown for many plant invaders (Bossdorf et al., 2004). Our results are consistent with the hypothesis that the introduction of specialized herbivores through biological control may be associated with a reversal of
this effect of initial enemy release. The assumed underlying mechanism of this pattern is a trade-off between resource allocation to growth, maintenance, storage, reproduction and defence. The data are consistent with a trade-off between growth for resource competition against *K. macrantha* and defense or resistance to biological control, which we did not test directly in our study. This would indicate that such a trade-off leading to lower competitive ability under biological control management has evolved after only one to two decades (i.e. selection on the total biomass: $Q_{SCh} > F_{SCh}$), but additional bioassays are needed to substantiate such trade-offs. A very recent study found a higher defence level in *O. commun* infested *A. artemisiifolia* populations than uninfested populations in China (Wan et al., 2019), which is well in line with our findings. Our study does support the EICA hypothesis associated with invasion success (Blossey and Nötzold 1995, Bossdorf, 2013), however, the results also show that the initially evolved increased competitive ability after the introduction into a new region can also be lost again quickly with the re-association of enemies, in this case specialize herbivores introduced for biological control (Müller-Schärer et al., 2004, Joshi and Vrieling, 2005).

We acknowledge a limitation of this part of our study as we observed only two CN populations without a history of biological control. Furthermore, these two CN populations are both from Northern China, thus differences between populations with and without biological control history could also be simply due to climate differences. In fact the Northern China region is unsuitable for the two biological control agents as shown in Sun et al. (2018). We found that the competitive ability of CN populations was affected by both biological control history, climate conditions, and their interactions. Yet, we found no significant $Q_{SC} > F_{SC}$ among all Chinese populations and no traits to be correlated with CN climate. Interestingly, total biomass becomes significantly correlated with climate for CN populations when we exclude the populations without biological control history (Fig. S3), which is consistent with observations of the US populations, i.e. the farther north the smaller the plant size. The fact that the two northmost populations without biological control history were slightly larger than the other CN populations and thus eliminated the climate-plant size relationship when considering all CN populations, further supports the role of adaptation in escaping from specialist
herbivores in the two CN populations without biological control history. Additional studies are clearly needed to disentangle the effects of enemy release and climate.

CONCLUSION

Species invasions are increasingly recognized as model systems for studying adaptive evolution over contemporary time scales due to demographic events/perturbations (e.g., founder effects, multiple introductions, admixture) and selection (Keller and Taylor, 2008). In A. artemisiifolia, for example, Gladieux et al. (2011) found high genetic diversity in the introduced French populations due to a mixture of source populations. Previous studies provided evidence for genetic differentiation in life-history traits (i.e. growth, reproduction), and potential adaption to local environmental conditions in the introduced French and the native US A. artemisiifolia populations (Hodgins and Rieseberg, 2011, Chun et al., 2011). Our results further reveal the signature of evolution for growth traits and phenotypic plasticity between native US and introduced CN regions of A. artemisiifolia and adaptation in traits that are expected to have favoured the invasion in China. Furthermore, we found different adaptive trajectories between the introduced CN populations differing in their history of biological control with specialized herbivores. A recent study on the invasive plant Impatiens glandulifera suggested that the recolonization of herbivores in introduced populations might select for its recovery in initially reduced levels of defence to pre-introduction levels over time (Gruntman et al., 2017). Such findings are in line with our results that natural enemies are likely to select and reduce the invasiveness related traits of A. artemisiifolia in the introduced CN region (Müller-Schärer et al., 2004, Müller-Schärer et al., 2014). Further studies involving bioassays and metabolomic analyses are required to assess whether the observed decreased competitive ability in the CN populations with biological control history is indeed associated with increased herbivore resistance.

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AUTHOR CONTRIBUTION

The main ideas were conceived by Y.S. and discussed with G.R.; Y.S. conducted the experiments, collected and analysed the data, and drafted the manuscript; G.R. provided suggestions and edited the manuscript.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.6039339

SUPPORTING INFORMATION

Table S1. Collection localities of Ambrosia artemisiifolia in China and the United States.

Table S2. Heritability ($h^2$) of phenotypic traits and plasticity indices for Ambrosia artemisiifolia populations.
Table S3. Chi-square and $P$-value of the maximum likelihood fitted LMMs/GLMMs of the effects of region, population, nutrient on phenotypic traits and plasticity indices for *Ambrosia artemisiifolia* populations.

Table S4. Variance components and $Q_{CT}$ values for all traits and plasticity indices of *Ambrosia artemisiifolia* populations from both China and the United States.

Table S5. Chi-square and $P$-value of the maximum likelihood fitted LMMs/GLMMs of the effects of population, nutrient on phenotypic traits and plasticity indices of *Ambrosia artemisiifolia* populations from the United States.

Table S6. Chi-square and $P$-value of the maximum likelihood fitted LMMs/GLMMs of the effects of population, nutrient on phenotypic traits and plasticity indices of *Ambrosia artemisiifolia* populations from China.

Table S7. Variance components and $Q_{SC}$ values for all traits and plasticity indices of *Ambrosia artemisiifolia* populations from China and the United States.

Table S8. Chi-square and $P$-value of the maximum likelihood fitted GLMMs/LMMs of the effects of biological control history, population, nutrient on phenotypic traits and plasticity indices of *Ambrosia artemisiifolia* populations from China.

Table S9. Variance components and $Q_{SC,h}$ values for all traits and plasticity indices of *Ambrosia artemisiifolia* populations from China with different biological control histories.

Figure S1. Comparison between three phenotypic traits and two phenotypic plasticity indices that showed significant $Q_{CT} > F_{CT}$ between invasive China populations with native United States populations of *Ambrosia artemisiifolia* under control and rich nutrient conditions.

Figure S2. Relationship between climate and latitude of all *Ambrosia artemisiifolia* populations from both China and United States.

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**Figure S3.** Relationship between climate and traits of *Ambrosia artemisiifolia* from Chinese (CN) populations with biological control history (subset of all CN populations in Fig. 4). Negative values for CN Climate PC1 indicate less seasonality and warmer summers.

**Figure S4.** Total biomass (left) and plasticity index of flower shoot number in response to nutrient addition (right) for invasive Chinese *Ambrosia artemisiifolia* populations with different biological control histories. The traits of total biomass also showed significant $Q_{SCA} > F_{SCA}$ (Fig. 5); error bars represent the ±SE. All data were transformed.

**Figure S5.** Relationship between China climate (CN Climate PC1) and reduction in biomass of *Koeleria macrantha*. Negative values for CN Climate PC1 indicate less seasonality and warmer summers (lower latitude). Open points and dashed line indicate the regression of reduction in biomass of *K. macrantha* when in competition with CN *Ambrosia artemisiifolia* populations with biological control history and grey points indicate those in competition with CN populations without biological control history.

**REFERENCE**


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**FIGURE LEGENDS**

**Figure 1.** Map of ten *Ambrosia artemisiifolia* populations sampled in the native (United States) and invasive (China) regions. Population details are given in Table S1.

**Figure 2.** Differentiation at quantitative traits among populations of *Ambrosia artemisiifolia* within each region (*Q*<sub>SC</sub>) and 95% bootstrap confidence limits (horizontal solid lines) for three growth traits, three reproduction traits, a phenology trait and six phenotypic plasticity indices indicated by points; differentiation at neutral genetic loci among populations within each region (*F*<sub>SC</sub>) and 95% bootstrap confidence limits (vertical dash line) indicated by vertical solid lines for the United States (a) and China (b), respectively. Probability: *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

**Figure 3.** Regional differentiation at quantitative traits among populations of *Ambrosia artemisiifolia* (*Q*<sub>CT</sub>) and 95% bootstrap confidence limits (horizontal solid lines) for three growth traits, three reproduction traits, a phenology trait and six phenotypic plasticity indices indicated by points; regional differentiation at neutral genetic loci (*F*<sub>CT</sub>) and 95% bootstrap confidence limits (vertical dashed lines) indicated by vertical solid lines for the United States vs. China. Probability: *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

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Figure 4. Relationship between climate and traits of *Ambrosia artemisiifolia* under selection from United States (US, solid black points) to China (CN, open points). Negative values for US Climate PC1 indicate more seasonality and cooler summers (higher latitude), while for CN Climate PC1 indicate less seasonality and warmer summers (lower latitude). Grey points indicate CN populations without biological control history.

Figure 5. Differentiation at quantitative traits between populations of *Ambrosia artemisiifolia* with vs. without biological control in China ($Q_{SC-h}$) and 95% bootstrap confidence limits (horizontal solid lines) for three growth traits, three reproduction traits, a phenology trait and six phenotypic plasticity indices indicated by points; differentiation at neutral genetic loci between populations with vs. without biological control in China ($F_{SC-h}$) and 95% bootstrap confidence limits (vertical dash line) indicated by vertical solid lines for populations with vs. without biological control in China. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Figure 6. Reduction in biomass of *Koeleria macrantha* when grown with the populations of Chinese *Ambrosia artemisiifolia* with different biological control history, compared to control pots; error bars represent the ±SE; the scale of the y-axis starts at 70% for greater clarity (a). Overall relationship between the biomass (gram, ln transformation) of *A. artemisiifolia* and that of *K. macrantha* in competition pots; each point represents a pot (b). Two colours of points represent the CN populations with/without biological control history.
Fig. 1
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Fig. 3
Fig. 4

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Fig. 6

The figure shows the reduction in biomass of Koeleria macrantha (in %) and the biomass of Ambrosia artemisiifolia (ln) with and without biocontrol history. The graph on the left displays the bar chart, where the height of the bars represents the percentage reduction in biomass. The graph on the right is a scatter plot with a trend line, indicating a negative correlation between biomass reduction and Ambrosia artemisiifolia biomass. The equation of the trend line is given as $R^2 = 0.2$ and $P = 0.006$.