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

KEYWORDS
adaptive divergence, alien invasive species, biological control, China, past selection, Q_{ST}-F_{ST}, USA

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1 | 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 (Bossdorf et al., 2005; Prentis, Wilson, Dormontt, Richardson, & Lowe, 2008). For example, Shirk, Hamrick, Zhang, and Qiang (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 contrary, genetic diversity can be higher in introduced as compared to native populations due to multiple introductions from genetically differentiated native populations (Genton, Shykoff, & Giraud, 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 & Taylor, 2008). Indeed, much work suggests adaptive change of invasive species to be common in novel environments (Phillips, Brown, Webb, & Shine, 2006; Prentis et al., 2008; Sakai et al., 2001).

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, Huey, Balanyà, Pascual, & Serra, 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, Elmendorf, & Vilà, 2007) and *Eschscholzia californica* (California poppies) (Leger & Rice, 2007). Further, there is growing evidence that alien invaders often show divergence from their native populations in traits that increase their invasiveness (Colautti & Lau, 2015; Hodgins & 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 & 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 favours genotypes with improved competitive ability at the expense of reduced herbivore defence, as defence against specialist herbivores no longer increases fitness (Blossey & Nötzold, 1995). This trade-off can be examined by comparing native and introduced populations (Bossdorf, Prati, Auge, & Schmid, 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, Steinger, Treier, & Mueller-Schaerer, 2008). Differences in phenotypic plasticities of invasive species between their native and introduced ranges are well studied and documented (Bossdorf et al., 2005; Chen, Berlocher, Opp, & Roderick, 2010; Hahn, Kleunen, & Müller-Schärer, 2012), yet less is known whether the plastic responses of traits is the result of adaptation to novel environments (van Kleunen, Schlaepfer, Glaettli, & Fischer, 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, Schmitt, Holbrook, Abu-Saba, & Flegal, 1994). 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, Lafferty, Dobson, McKenzie, & Kuris, 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 (DeWoody et al., 2010; Leinonen, McCairns, O'Hara, & Merilä, 2013; Leinonen, O'Hara, Cano, & Merilä, 2008; Steinger, Haldimann, Leiss, & Müller-Schärer, 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 & Lau, 2015; Müller-Schärer, Schaffner, & Steinger, 2004; Xu et al., 2010). Such evolutionary changes can occur not only between the native and introduced regions but also within the introduced region over time (Gruntman, Segev, Glauser, & Tielbörger, 2017; Phillips et al., 2006), such as for organisms introduced for biological control (Roderick, Hufbauer, & Navajas, 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 & Maron, 2006; Roderick et al., 2012). Using a joint approach of population genetics and two common garden experiments, we address the following questions: (a) 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? (b) Is climate a possible driver of evolution between two regions? (c) Do introduced populations show increased phenotypic plasticity for relevant traits, such as

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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.

2 | MATERIALS AND METHODS

2.1 | 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, Guan, & Wan, 1993), and has by now expanded to 21 provinces in northern, central, eastern and southern China (Zhou, Guo, Chen, & Wan, 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, Li, Gregg, & Li, 2001).

2.2 | 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 programmes 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 (S. S. Zhou, pers. comm.).

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 (Figure 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 *A. artemisiifolia* is predominantly outcrossing, with very high outcrossing rates (0.93–1.0) for both Chinese and North American populations (Friedman & Barrett, 2008; Li, Liao, Wolfe, & Zhang, 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 (Figure 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-hr darkness) for 4 weeks to break primary dormancy (Willemsen, 1975).

2.3 | 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-hr day regime with 20°C day and 10°C night temperature to simulate early spring conditions (Leiblein-Wild, Kaviani, & Tackenberg, 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 2 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 DNesay™ 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,

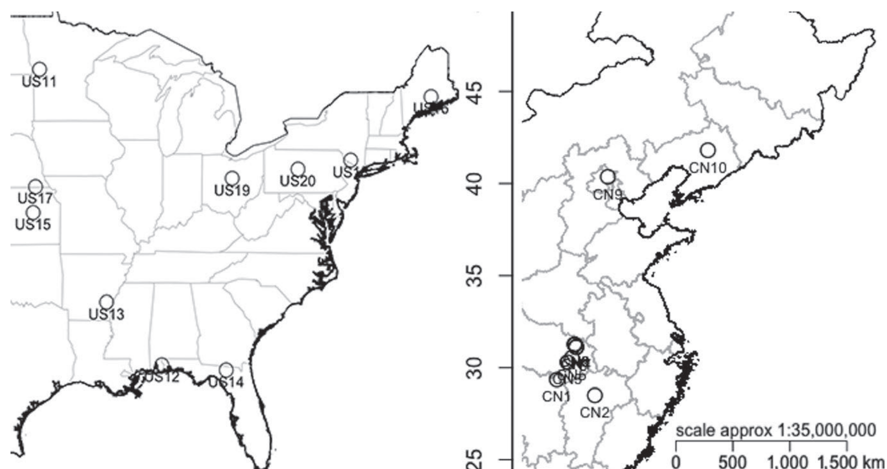

FIGURE 1 Map of 10 *Ambrosia artemisiifolia* populations sampled in the native (United States) and invasive (China) regions. Population details are given in Table S1

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AY849312, Genton et al. (2005); *Ambart 04*, *06*, *09*, *17*, *18*, *21*, *24* and *27*, GenBank accession no: FJ595149–FJ595156, Chun, Fumanal, Laitung, and Bretagnolle (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) 18 bp universal sequence (5-TGT AAA ACG ACG GCC AGT-3) with the original primer sequence (Schuelke, 2000). All primers and a FAM-labelled 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 $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 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, Corre, & Bretagnolle, 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, USA).

Descriptive statistics for microsatellites including observed heterozygosity (H_o), expected heterozygosity (H_e), observed gene diversities (H_s), overall gene diversity (H_t), and inbreeding coefficients (F_{IS}) were estimated using the R package *adegenet* (Jombart, 2008) based on the allele designation from fragment size determinations and *diveRsity* with respective 5,000 bootstrapped iterations to execute 95% confidence intervals (Keenan, McGinnity, Cross, Crozier, & Prodöhl, 2013). Multilocus variance components and *F*-statistics were calculated using the package *hierfstat* in R (Goudet, 2005). F_{ST} over regions and populations within regions were estimated by permutation tests, with 95% confidence intervals by the range of the central 95% of 5,000 bootstrap estimates.

2.4 | 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, Bossdorf, Muth, Gurevitch, & Pigliucci, 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 2 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-hr light, 6-hr 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 72 hr to a constant weight at 80°C and weighed to an accuracy of ±0.001 g. 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 $\mathrm{PI} = \frac{F_N - \overline{F_C}}{F_N}$, where F_N is the trait value of each individual plant with additional nutrient treatment, and $\overline{F_C}$ 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.

2.5 | 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, Bates, DebRoy, Sarkar, & Team, 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 ($h^2_N = \frac{V_A}{V_A + V_E}$) for all seven phenotypic traits and six PIs. The within-family variance, σ^2_E, estimated the environmental variance, V_E, and the genetic variance due to residual additive genetic factors, σ^2_G, 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 σ^2_G is constrained to zero with that of a model in which σ^2_G 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 ($Q_{SC\text{-}h}$) were calculated as $Q_{CT} = \frac{\sigma_R^2}{\sigma_R^2+\sigma_P^2+2\sigma_F^2}$, $Q_{SC} = \frac{\sigma_P^2}{\sigma_P^2+2\sigma_F^2}$ and $Q_{SC\text{-}h} = \frac{\sigma_H^2}{\sigma_H^2+\sigma_P^2+2\sigma_F^2}$, respectively, where σ_R^2 is the additive genetic variance between-region, σ_P^2 is the additive genetic variance among-population, σ_H^2 is the additive genetic variance between different biological control history within CN and σ_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 & 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 σ_F^2, therefore, may be inflated, biasing our Q_{CT}, Q_{SC} and $Q_{SC\text{-}h}$ estimates downward (Lynch & 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 5,000 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 $Q_{SC\text{-}h}$

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