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Genetic differentiation in life-history traits of introduced and native common ragweed (*Ambrosia artemisiifolia*) populations

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herbivory;
life-history trade-offs;
light stress.

Abstract

Introduced species represent opportunities to observe evolution over contemporary time scales, and as exotics encounter new environments, adaptive responses can occur, potentially contributing to invasion. Here, we compare 22 native North American populations and 12 introduced European populations of common ragweed (*Ambrosia artemisiifolia*) in five common gardens (control, herbivory, light stress, nutrient stress and drought). We found evidence for improved growth and reproduction of the introduced populations in most environments, particularly in the light stress. However, under drought conditions, the introduced plants experienced more rapid wilting and mortality than their native counterparts, evidence consistent with a life-history trade-off between rapid growth and drought tolerance. Moreover, we found parallel latitudinal clines in flowering time and correlations between fitness components and the local climate of the source populations in both ranges. Together these data provide evidence for adaptation to local environmental conditions in the native and introduced range of common ragweed.

Introduction

Weedy and invasive plants represent a major economic and environmental concern. Annual economic costs in North America are estimated at C$30–40 billion (Pimentel *et al.*, 2000; Myers & Bazely, 2003; Colautti *et al.*, 2006a), and in addition to their direct economic damage, through such costs as lost crop production and the implementation of control measures, invasive plants also threaten native biodiversity and disrupt ecosystem function (McNeely, 2001; Colautti *et al.*, 2006a). Consequently, the substantial ecological and financial burdens caused by invasive species provide considerable incentive for understanding how plants become invasive in order to develop more effective methods to prevent and control invasions. However, despite a plethora of research into invasion biology, our understanding of the biological mechanisms that allow certain species to become so abundant in their introduced ranges is limited.

There is a large body of research devoted to the ecological factors contributing to plant invasion (Kolar & Lodge, 2001; Myers & Bazely, 2003; Colautti *et al.*, 2006b). However, more recently, there has been growing interest in studying biological invasion in an evolutionary context (Lee, 2002; Allendorf & Lundquist, 2003; Dlugosch & Parker, 2008a,b). Exotic species must cope with new biotic environments (Williamson, 1996) and perhaps different abiotic conditions (climatic or edaphic). Consequently, a species' success in a new range may depend on its ability to evolve in response to a different selective regime compared with that of its native range. Indeed, there are a growing number of studies demonstrating rapid evolutionary change during expansion into an introduced range, suggesting that invasions represent promising systems for studying adaptation over contemporary time scales (Baker & Stebbins, 1965; Sakai *et al.*, 2001; Lee, 2002; Dlugosch & Parker, 2008b; Keller & Taylor, 2008).

A change reported in some alien plants is increased growth and reproduction (Elton, 1958; Crawley, 1987; Thébaud & Simberloff, 2001), and such improved vigour could contribute to the rapid spread of these species in the introduced range. Although increased size is not a

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ubiquitous feature of plant invaders (e.g. Willis *et al.*, 2000; Thébaud & Simberloff, 2001), there have been several hypothesis put forth to explain its occurrence in species where it has been observed. The most well known of these is the evolution of increased competitive ability hypothesis (EICA). This hypothesis posits that some invaders have escaped from biotic enemies, such as specialized herbivores, pathogens and competitors found in their native ranges (Blossey & Notzold, 1995; Keane & Crawley, 2002; Callaway & Ridenour, 2004), and resources formerly allocated to costly self-defence mechanisms are instead reallocated to other functions such as increased growth, competitive ability or reproduction. Trade-offs between enemy resistance and tolerance, and growth or reproduction have been observed in many plants (reviewed by Herms & Mattson, 1992) including several invasive species (Blair & Wolfe, 2004; Rogers & Siemann, 2005; Huang *et al.*, 2010; but see Parker & Gilbert, 2007; van Kleunen & Schmid, 2003), suggesting that the changes in resource allocation in response to herbivores could be contributing to the invasiveness of some species.

An alternative, but not mutually exclusive hypothesis is that some invasive species have switched from a strategy of high tolerance to abiotic environmental stress to a strategy of enhanced competitive or colonizing ability (Alpert *et al.*, 2000; Richards *et al.*, 2006; He *et al.*, 2010). This hypothesis assumes that trade-offs exist such that plants are unable to be both highly tolerant to abiotic stress and highly competitive (or reproductive) (Grime, 1977). There are many empirical studies that support the existence of such trade-offs. For example, plants that are highly tolerant to saline or toxic soils have been shown to compete poorly for more benign sites (e.g. Jurjavcic *et al.*, 2002; Crain *et al.*, 2004). Tolerance to other abiotic stress, such as drought, has been shown to come at the expense of increased growth rate and reduced competitive ability in several species (e.g. Aronson *et al.*, 1992; Petrů *et al.*, 2006; Sambatti & Rice, 2007; Liancourt & Tielbörger, 2009). Therefore, introduced populations experiencing less extreme abiotic environments could evolve along such trade-offs, and this could contribute to increased vigour experienced by some invasive species in their nonindigenous ranges.

Under either of these hypotheses, we would expect invasive and weedy species to have evolved a lower tolerance to certain extreme and stressful conditions. Under the first hypothesis, invasive species are expected to gradually lose resistance or tolerance to enemies or competitors present in their native environment due to their absence in the introduced range. Under the second hypothesis, invasive and weedy species are expected to have lost resistance or tolerance to abiotic stress as they evolved a life-history strategy that specializes on rapid growth and reproduction. Other factors could also contribute to genetically based improvements in growth and reproduction in invasive populations besides changes in resource allocation. For example, hybridization in the invasive range, either interspecific or among previously isolated populations of the same species, has been documented in a number of invasive species (e.g. Ellstrand & Schierenbeck, 2000; Gaskin & Schaal, 2002; Kolbe *et al.*, 2007; Zalapa *et al.*, 2010) and could contribute to invasiveness through hybrid vigour and the formation of novel genotypes. Increased genetic variation due to such admixture could provide the fuel for rapid adaptive evolutionary change during invasion.

Common ragweed (*Ambrosia artemisiifolia*) is an aggressive annual weed native to North America and has been introduced into parts of Australia, Asia and Europe. The weed is particularly problematic in France and in many eastern European countries, such as Hungary where it is very abundant, reaching high population densities (Chauvel *et al.*, 2006; Kiss & Beres, 2006). Two lines of evidence support a complex invasion history characterized by genetic admixture of divergent native common ragweed populations in Europe. First, historical records indicate that the invasion of common ragweed was facilitated through the repeated import of contaminated North American horse-fodder during World War I. Contaminated birdseed, potatoes, wheat and red clover seeds from North America are other documented sources of ragweed introduction into Europe (Chauvel *et al.*, 2006). Second, genetic evidence suggests that European populations are a result of multiple introductions from North America (Genton *et al.*, 2005a; Chun *et al.*, 2010; Chun *et al.*, 2011; Gaudeul *et al.*, 2011). For example, studies of microsatellite variability have found higher within-population genetic variability in France than in eastern North America (Genton *et al.*, 2005a), and assignment tests suggest that European populations were sourced from many North American populations (Genton *et al.*, 2005a; Gaudeul *et al.*, 2011). Taken together, the historical accounts and genetic data provide strong evidence for multiple introductions at a local and regional scale in Europe.

Our goal is to determine whether there is any genetically based phenotypic differences in growth and reproduction between native and introduced populations of common ragweed that could contribute to its invasiveness in Europe. Specifically, we addressed two questions. First, do introduced populations exhibit greater growth and reproduction in benign conditions compared with native populations? Second, do introduced populations lose any fitness advantage under certain stressful conditions, which could suggest the presence of trade-offs? To answer these questions, we performed a series of common garden glasshouse experiments using seeds gathered from 12 introduced populations from Europe and 22 native populations from North America. These experiments allowed us to determine whether there is any evidence for differences in resource allocation between native and introduced common ragweed that may have evolved during the expansion of the species out of its indigenous range. Moreover, the use of common gardens and the experimental application of the stresses allowed us to identify specific environmental factors that may be responsible for any differences that we observed.

Methods

Study species

Common ragweed (*Ambrosia artemisiifolia*) is an erect annual herb typically found in disturbed habitats, such as abandoned and cultivated fields and along rivers and roads (Bassett & Crompton, 1975). It is a well-known agricultural weed and produces highly allergenic pollen (Bassett & Crompton, 1975; Laaidi *et al.*, 2003). The species typically prefers full sun and average to slightly dry conditions and is found in a wide variety of soil types, but will thrive in soil containing high amounts of clay, gravel or sand because of reduced competition from other plants. It is a self-incompatible (Friedman & Barrett, 2008), monecious, wind-pollinated species. It has uniovulate flowers, but a large individual may produce more than 60 000 seeds (Dickerson & Sweet, 1971). Seed dispersal by water, birds and humans is likely important for the spread of common ragweed, although the seeds possess no obvious dispersal mechanism (Bassett & Crompton, 1975). Common ragweed is considered a persistent and aggressive weed because the seeds can remain viable in the soil for several years.

Seed collections

We used seed material collected from North American and European populations during the fall of 2008. We obtained maternal seed families (approximately 30/population) from 12 populations in Europe (introduced range) and 22 populations in North America (native range) for a total of 34 populations (Fig. 1). For two native populations (AA18 and AA19), germination and survival were low and they were removed from some analyses. We recorded the latitude and longitude for each population (Table S1). Pollen cores indicate that *Ambrosia* spp. have been abundant in central North America for the past 15 000 years but relatively rare elsewhere in the continent until modern times (Williams *et al.*, 2004). It is unclear whether common ragweed is native to eastern North America or whether it has been introduced from the prairies (Bassett & Crompton, 1975; Lavoie *et al.*, 2007). However, pollen cores and herbarium records indicate that this species has become substantially more abundant in eastern North America over the past 200 years due to increased large-scale disturbance and the growth of agriculture (Lavoie *et al.*, 2007). As ragweed species have been found at high relative

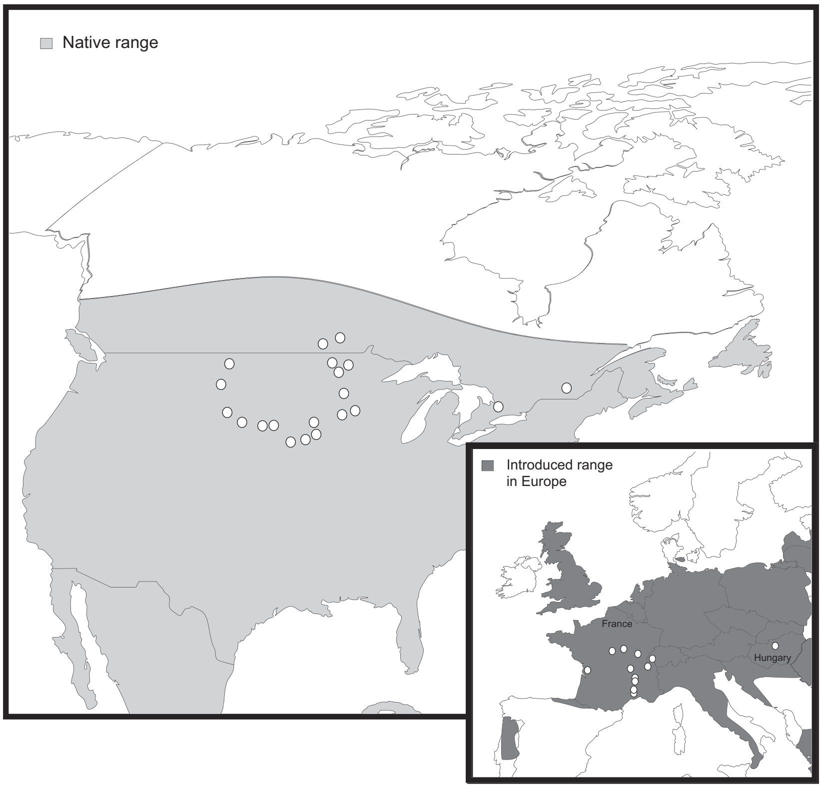

Fig. 1 The location of the populations of common ragweed (*Ambrosia artemisiifolia*) that were sampled for the common garden experiments, as well as the distribution of common ragweed in North America (native range after Genton *et al.*, 2005a) and in Europe (introduced range after ''DAISIE European Invasive Alien Species Gateway,'' 2008. Retrieved April 2011, from http://www.europe-aliens.org/speciesFactsheet.do?speciesId=21692). In total, 22 populations were sampled in North America and 12 populations were sampled in Europe (denoted by open circles).

abundance in the central region of North America for thousands of years in the absence of significant human disturbance, we sampled the majority of the 22 populations from this region rather than from eastern North America. Moreover, in the native range, we focused our sampling efforts on low-density common ragweed populations, which is typical of populations from the Great Plains region (personal observation), whereas in Europe, we sampled large, high-density invasive populations.

Common garden experiment

Following stratification procedures suggested in Willemsen (1975), we sowed 12 seeds from seven maternal families from each native population and ten maternal families from each introduced population on damp filter paper in petri dishes with 1% plant preservative mixture. We placed the seeds in a germination chamber with a 24 °C day and a 18 °C night and a 14:10-hr light/dark cycle. Prior to germination, we weighed 12 seeds from each family and then averaged the seed mass for each family. On June 8 and 9, 2009, after 5–6 days in the germination chamber, germination rates were recorded. Depending on the number of seeds that germinated in each family, four to six seedlings were randomly selected and transplanted into one-inch pots that were filled with ½ sand and ½ potting soil and were misted at regular intervals. The remaining seeds were monitored for another week, and no further germination occurred after this point. All plants were grown at the University of British Columbia's Horticulture Building.

After 2 weeks, we transplanted the seedlings to 3½ -inch pots with the same sand/soil mixture. We added 1.5 mL of 13:13:13 Osmocote® (Scott-Sierra, Marysville, OH) slow release fertilizer to all pots with the exception of the nutrient stress treatment. All plants were watered by automatically flooding the bench, and the frequency and duration of watering varied depending on the size of the plants and the temperature of the glasshouse. Before transplanting into these larger pots, we recorded plant height and leaf number (time-point T_1) and randomly assigned the plants from each family to a treatment. One individual from each family was assigned to each of three treatments (light stress, simulated herbivory and nutrient stress) and a control, and any additional plants were also placed in the control. We then divided the glasshouse bench into three blocks, and families were randomly assigned to each block. Each of the three stress treatments and the control was assigned in a random order to each block. Within each block and treatment combination, individuals were randomly assigned to a tray. The position of an individual within the tray changed weekly, and the location of the tray within each block and treatment combination was randomized every 2 weeks.

After 2 weeks, we measured the maximum width and height of all plants (time-point T_2) and applied the light stress and simulated herbivory stress. To simulate above-ground neighbour effects, we constructed three shade boxes (1.5 × 0.6 × 1.3 m) with PVC piping. We placed green filters (Lee, Andover, UK: number 121 Lee green) and neutral density shade cloth around all five sides of the PVC frame. We used the green filter to mimic the spectral quality of light that is transmitted through the leaves of plants (Bonser & Aarssen, 2003) and the shade cloth to further reduce the quantity of light. The green filter reduced the light transmittance by 73%, and the shade cloth further reduced transmittance to 92% of the original value (light intensities were reduced from an average of 873.3–66.1 μmol m^{-2} s^{-1} based on an average of three measurements taken at noon on sunny days). We simulated herbivory using mechanical damage and the application of 1 mM of methyl jasmonate (MeJA). When plants are exposed to volatile MeJA, they elicit a series of defence responses, and MeJA treatment is a commonly used means of eliciting a herbivore response in many different plant species (McConn *et al.*, 1997; van Dam & Baldwin, 1998; Li *et al.*, 2002). Each week we removed half of each new fully expanded, undamaged leaf >2 cm in any dimension, so that each leaf was only cut once, and then sprayed each plant with MeJA until each leaf was soaked. During the MeJA treatment, the plants were removed from the bench and left to dry to avoid any transfer of the MeJA to the other experimental plants.

Two weeks (time-point T_3) and 6 weeks (time-point T_4) after the application of the light and herbivory stresses, we measured the height and maximum stem diameter at the base of the stem of all plants, as well as the maximum plant width and maximum leaf length of all individuals, except for those from the simulated herbivory treatment, as the mechanical damage would bias these measurements. At the commencement of flowering in July, we examined the plants every 2–3 days and recorded the first day of male and female flowering for each plant until the end of the experiment in mid-October 2009. Just before the end of the experiment, when approximately half of the plants in the control were beginning to show signs of senescence, we measured height, stem diameter and branch number, defined as the number of branches extending from the main stem, from all individuals (time-point T_5). We also recorded the maximum width and leaf length from all plants except those from the herbivory stress. We recorded two binary responses as well: (i) if an individual survived from time-point T_1 to the end of the experiment and (ii) if an individual flowered before the end of the experiment. We then removed and counted the number of all male inflorescences (capitula) and removed all seeds and female flowers from each individual. We dried the male and female reproductive biomass, as well as the above-ground vegetative biomass for approximately 2 weeks in a 60 °C oven, until the vegetative biomass did not decline more than 0.01 g over 24 h. We then weighed the male and female reproductive biomass, as well as the above-ground vegetative biomass. For 113 individuals, we recorded the total number of seeds and female flowers to determine how well female reproductive biomass could statistically predict the total number of female flowers produced in each treatment, as counting the total flower production was prohibited by time.

Common garden experiment data analysis

We conducted all statistical analysis using SAS (Version 9.2; SAS Institute, Inc., Cary, NC, USA). To assess whether there were differences in early acting components of fitness, average seed mass, germination rate, height and width (T_1 and T_2), as well as leaf number (T_1), between the native and introduced populations, we first performed a MANOVA on populations means (PROC GLM). As the use of highly correlated variables in MANOVA can reduce the power of the analysis (Scheiner, 1993), we removed traits that were highly correlated ($R > 0.80$) with other traits in the analysis (height T_2 and width T_2). We then examined the standardized canonical coefficients to identify the unique contribution of each variable to the differences between the groups.

We then examined each trait independently in individual univariate analyses. We first examined germination rates and average seed mass for each maternal family. Average seed mass for each family was analysed using PROC MIXED, and the proportion of seeds germinating for each maternal family was analysed using PROC GLIMMIX with a binomial distribution and a logit link. For both analyses, range (native or introduced) was the fixed effect and population nested within range was included in the model as a random effect. Similarly, initial leaf number, height at transplant (time-point T_1) and height and width measured just prior to the start of the light and herbivory stress (time-point T_2) were analysed using mixed models. Nutrient stress plants were removed from the analysis at time-point T_2, as the nutrient stress began at the time of transplant (T_1) and even a short time without fertilizer could influence growth. Range was included in the model as a fixed effect, and population nested within range and family nested within population and range were included in the model as random effects. For time-point T_2, block was also included in the analysis as a random effect. PROC MIXED was used to analyse the height and width data. For all analyses, the continuous response variables were transformed as needed (square-root or log-transformed) to improve normality and reduce heteroscedacity of the data. A generalized linear mixed model (PROC GLIMMIX) was used for leaf number with a Poisson-distributed response and a log link.

To compare the differences in growth and reproduction between introduced and native populations after the start of the stress, we initially conducted a MANOVA on population means (PROC GLM) with range, treatment, as well as their interaction as fixed effects. The dependent variables included in the analysis were height, stem diameter, reproductive biomass, branch number, proportion surviving and proportion flowering measured at the end of the experiment (time-point T_5). Only measurements taken at the final time-point were included due to correlations of traits among time-points. A second analysis was carried out excluding the herbivory treatment, but including width and provided similar results, so only the first analysis is presented. Also, as width and leaf length, as well as stem diameter and vegetative biomass, were highly correlated ($R > 0.80$), we only incorporated width and stem diameter in the MANOVAS. Significant interactions between range and treatment were further dissected using contrasts comparing native and introduced populations in each treatment, and a sequential Bonferroni correction was applied.

We then performed mixed model analyses (PROC MIXED) on each trait individually. We included range, treatment, as well as their interaction, as fixed effects. We also included block, population nested within range, maternal family nested within block, population and range, as well as all possible two-way and three-way interactions. We excluded all random interaction terms from the model by backward elimination if they did not explain a significant proportion of the variation in the dependent variable ($\alpha = 0.10$). We retained the interaction between range and treatment because it was important to our experimental design, as a significant interaction could indicate the presence of a trade-off. Our response variables were height, maximum width, maximum leaf length and stem diameter. These variables were measured at all three time-points (T_3, T_4 and T_5). In addition, we also analysed branch number, total above-ground biomass and reproductive biomass of flowering individuals (total as well as male and female reproductive biomass), which were recorded at the end of the experiment (T_5). We analysed the binary response variables survivorship and flowering using generalized linear mixed models for categorical data using a logit link (PROC GLIMMIX) using the same model as described earlier.

For the mixed models, we tested for heterogeneity of variance between the ranges and among the treatments using Levene's test (PROC GLM). If significant heterogeneity of variance occurred for these effects and could not be reduced by transformation, we estimated residual variances for each group (Littell *et al.*, 2006). The models with unequal variances were compared with models with equal variances with the Akaike's information criterion, to further confirm whether a more complex covariance structure was required.

For all mixed models, we calculated denominator degrees of freedom for *F*-tests by Kenward & Roger's (1997) approximation, which can result in fractional degrees of freedom. For general linear mixed models, the significance of random effects was assessed using likelihood ratio tests by comparing −2 log-likelihoods between the full model and a reduced model without the random effect. The difference in the −2 log-likelihoods should be distributed as a χ^2 variable with a single degree of freedom (Littell *et al.*, 2006). To test for the significance of random effects for generalized linear mixed models, we used the COVTEST statement in PROC GLIMMIX, which provides statistical inferences for the covariance parameters by likelihood-based tests (including pseudolikelihood methods) comparing full and reduced models with respect to the covariance parameters. Significant differences between the treatments or between ranges within treatments were assessed using differences of least squares means, and the Tukey–Krammer adjustment was used to correct *P*-values for multiple comparisons (Kramer, 1956). We show least squares means, which are adjusted means for unbalanced designs, and standard errors, and back-transform means and standard errors where necessary for presentation in the figures or tables.

To assess the importance of bioclimatic variation in driving differences in the reproductive and vegetative traits of the native and introduced populations, we obtained 19 bioclimatic variables from the WorldClim database Version 1.4 (release 3) for each population (Hijmans *et al.*, 2005). Due to the number and the multicollinearity of the bioclimatic data, we used principal components analysis (PCA) to summarize the patterns of correlation between bioclimatic variables and performed PCA on the correlation matrix of these variables using PROC PRINCOMP to obtain the first principal component of the bioclimatic data ($\mathrm{PC1_{bio}}$). We then performed a MANCOVA using the same dependent and independent variables listed earlier for the early (T_1) and final measurements (T_5), but also including either latitude (decimal degrees) or $\mathrm{PC1_{bio}}$ as covariates in an analysis of the population means. For the final measurements, a third MANCOVA was performed using initial leaf length (T_1) to control for initial size differences before the application of the stress. Nonsignificant interactions (≤0.10) with the covariates were removed in a stepwise manner. We then performed univariate analyses for each trait. For most traits, we used general linear models (PROC GLM), the exceptions being the proportion of individuals that flowered and the proportion surviving from time-point T_1 until the end of the experiment for each population in each treatment, where we used PROC GENMOD with a binomial distribution and a logit link. If significant overdispersion was detected, we used the PSCALE option to estimate a scale parameter for the variance. The fixed effects in the analyses were treatment, region, covariate (latitude, $\mathrm{PC1_{bio}}$ or leaf length) and all interactions. Measurements taken prior to the start of the stress did not include treatment, interactions involving treatment or leaf length as a covariate in the analysis. Nonsignificant interactions (≤0.10) with the covariates were removed in a stepwise manner starting with the highest-order interaction. As mentioned earlier, significant differences between the treatments or between the ranges within treatments were assessed using differences of least squares means, and the Tukey–Krammer adjustment was used to correct *P*-values for multiple comparisons (Kramer, 1956).

Drought experiment

On April 3 and 4, 2009, we sowed out six seeds from each of ten maternal families from all European populations and seven maternal families from all North America populations. We followed the same germination and transplant procedure as mentioned previously. We randomly assigned one individual from each family into trays. Due to variable germination and survival prior to the start of the experiment, our final sample size was 90 native individuals and 90 introduced individuals. We rotated the position of the plants within the trays weekly and randomized the position of the trays every 2 weeks. We measured leaf number and height as well as maximum width and height at approximately the same stage as time-points T_1 and T_2. On May 13, 2009, we deprived the plants of water and recorded the number of days until wilting and the number of days until death. We took population medians for each trait and performed a survival analysis, so our survival analysis examined the number of days until half of the individuals in the population had either wilted or died. We first compared the introduced and native populations using a log-rank test (PROC LIFETEST). We then examined the association between survivorship and range, as well as plant height or width using cox regression (PROC PHREG).

Maternal effects experiment

We selected five European and five North American populations to determine the extent of maternal effects on early growth. On March 8, 2010, we sowed out six seeds from each of 10 maternal families per population from Europe and 10 maternal families per population in North America. A single representative of each family was randomly selected and planted. We followed the same germination and transplant procedure as mentioned earlier. Half of the plants within each population were randomly assigned as pollen donors, and the other half were reserved as maternal parents. Once the plants began to produce inflorescences, we placed the pollen donors in a separate glasshouse, emasculated all maternal plants and covered approximately 20–30 uniovulate flowers on each plant with three layers of spun-fibre material following Friedman & Barrett (2008). We randomly assigned flowering pollen donors to the maternal plants from the same population. We allowed the seeds to mature and collected them 2–4 weeks after pollination.

On October 29, 2010, we sowed out up to 6 seeds from each maternal family. We followed the same germination and transplant procedures as mentioned earlier. Plants were randomly assigned to trays, and trays were randomly placed on the bench and randomized once per week. We measured the maternal and offspring plants at the same stage as time-points T_1 and T_2 described earlier. Specifically, just prior to transplant, we measured leaf number and height (T_1), and 2 weeks later, we measured height and width (T_2). After these measurements were taken on the offspring generation, we ended the experiment, as the early growth measurements were indicative of later growth and reproductive success in all treatments in the previous experiment, and maternal effects are typically strongest during early life stages (for review, see Roach & Wulff, 1987).

Maternal effects analysis

For both generations, height at transplant (T_1) and height and width measured 2 weeks following transplant (T_2) were analysed using PROC MIXED. Random effects were assessed in the same manner as described earlier. Similarly, leaf number was analysed using PROC GLIMMIX, in the same manner as described earlier. Generation (maternal or offspring), range (introduced or native) and their interaction were included in the model as fixed effects, and population nested within range, and family nested within population and range were included in the model as random effects. Only maternal plants that produced offspring were used in the analysis. Therefore, four to seven maternal plants (mean = 5.1) were used from four native and five introduced populations, and from those, 22–34 (mean = 29.3) offspring per population germinated and were used in the experiment.

Results

Germination and early growth

As predicted, we found evidence for increased juvenile growth in the European populations compared with the North American populations. Results of the MANOVA of early growth traits indicated a significant effect of range (Wilks' lambda = 0.55, $F_{4,28} = 5.64$, $P < 0.01$) and standardized canonical correlation coefficients of −0.89 for height (T_1), 1.2 for leaf number (T_1), 0.22 for germination rate and 0.78 for seed mass. To summarize the MANOVA, plants sampled from the introduced range had greater leaf production, germination rate and seed mass but were of shorter stature than those from the native range when controlling for other dependent variables. A discriminate functions analysis of the same early growth traits using cross-validation (PROC DISCRIM) misclassified two of the 12 introduced populations from France as native (FR1 and FR4) and six of the 21 native populations as introduced (AA2, AA3, AA4, AA8, AA9 and MNON). This result implies some overlap between the ranges in the early growth traits, but there is no obvious geographical pattern regarding the origin of these overlapping populations (Table S1).

Univariate analysis provided similar results as the MANOVA. At the time of transplant (T_1), we found a significantly greater number of leaves in the introduced plants (Fig. 2a; range $F_{1,202} = 29.1$, $P < 0.001$), but no difference in height (range $F_{1,31} = 0.09$, $P = 0.77$). Unsurprisingly due to the high correlation between leaf number (T_1) and width (T_2), we also found that unstressed introduced plants had a significantly greater maximum width than native plants (Fig. 2b; range $F_{1,29} = 4.47$, $P < 0.05$). We observed no difference in plant height at time-point T_2 (range $F_{1,29.3} = 0.00$, $P = 0.99$). However, seed mass was not significantly greater in the introduced populations compared with the native populations (range $F_{1,28.9} = 0.13$, $P = 0.72$; introduced mean ± SE: 7.4 mg ± 2.9; native mean ± SE: 9.0 mg ± 1.9) nor was germination rate (range $F_{1,34.6} = 2.97$, $P = 0.09$; introduced mean ± SE = 0.77 ± 0.04; native mean ± SE = 0.68 ± 0.03). We found that the inclusion of population significantly improved the model fit for every early growth trait measured at T_2 ($P < 0.05$). Block and family were significant for width and height measured at time-point T_2 ($P < 0.05$), and the interaction between block and population was also significant for height at T_2 ($P < 0.05$).

The effects of nutrient, herbivory and light stress

MANOVA of the traits measured at the end of the experiment (T_5) indicated a significant treatment effect (Wilks' lambda = 0.02, $F_{18,334.24} = 52.24$, $P < 0.001$),

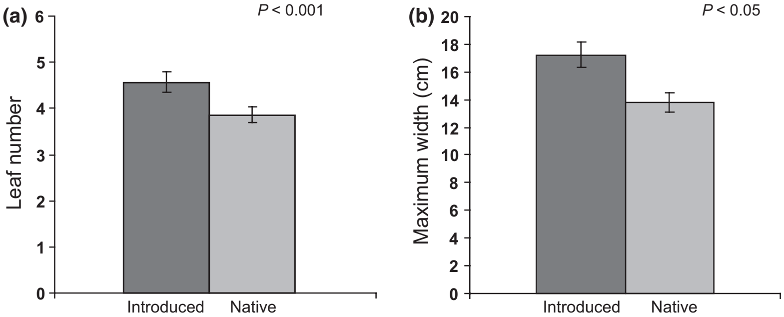

Fig. 2 Early size-related traits measured for native (North American) and introduced (European) common ragweed (*Ambrosia artemisiifolia*) grown in a common garden. Range (native or introduced) was the only fixed effect in both models. (a) The leaf number at the time of transplanting (T_1). (b) The maximum width prior to the application of the stress (T_2). Least squares means and standard errors are shown.

Table 1 MANOVA and MANCOVAS for native and introduced common ragweed (*Ambrosia artemisiifolia*) grown in four common gardens (nutrient stress, herbivory stress, light stress and control) for population means of growth and reproductive traits measured at the end of the experiments (height, stem diameter, reproductive biomass, proportion flowering and proportion surviving). Wilks' lambda (Λ) is provided for each effect. Contrasts for comparisons between native and introduced populations in each treatment are shown, and those contrasts still significant after a sequential Bonferroni are in boldface.

	Covariate			
Fixed effect	None	Leaf number	Latitude	PC1$_{bio}$
Treatment	Λ = 0.022 $F_{18,334.24}$ = 52.24***	Λ = 0.020 $F_{18,331.41}$ = 55.80 ***	Λ = 0.021 $F_{18,331.41}$ = 53.19***	Λ = 0.020 $F_{18,328.58}$ = 53.97***
Range	Λ = 0.63 $F_{6,118}$ = 11.36***	Λ = 0.74 $F_{6,117}$ = 6.94***	Λ = 0.65 $F_{6,117}$ = 10.67***	Λ = 0.86 $F_{6,116}$ = 3.25**
Range × treatment	Λ = 0.80 $F_{18,334.24}$ = 1.57†	Λ = 0.75 $F_{6,331.41}$ = 1.96*	Λ = 0.79 $F_{18,331.41}$ = 1.60†	Λ = 0.79 $F_{18,328.58}$ = 1.63†
Covariate	–	Λ = 0.65 $F_{6,117}$ = 10.11***	Λ = 0.81 $F_{6,117}$ = 4.72***	Λ = 0.90 $F_{6,116}$ = 2.05†
Covariate × range	–		–	Λ = 0.85 $F_{6,116}$ = 3.30**
Control	Λ = 0.80 **$F_{6,118}$ = 4.38*** **	Λ = 0.82 **$F_{6,117}$ = 4.24*** **	Λ = 0.81 **$F_{6,117}$ = 4.69*** **	Λ = 0.82 **$F_{6,117}$ = 4.69*** **
Herbivory	Λ = 0.78 **$F_{6,118}$ = 5.71*** **	Λ = 0.82 **$F_{6,117}$ = 4.41*** **	Λ = 0.78 **$F_{6,117}$ = 5.66*** **	Λ = 0.78 **$F_{6,117}$ = 5.66*** **
Light	Λ = 0.81 **$F_{6,118}$ = 4.48*** **	Λ = 0.84 **$F_{6,117}$ = 3.64** **	Λ = 0.82 **$F_{6,117}$ = 4.31*** **	Λ = 0.84 **$F_{6,117}$ = 4.31*** **
Nutrient	Λ = 0.94 $F_{6,118}$ = 1.17 (ns)	Λ = 0.94 $F_{6,117}$ = 1.33 (ns)	Λ = 0.95 $F_{6,117}$ = 0.39 (ns)	Λ = 0.95 $F_{6,117}$ = 1.07 (ns)

ns $P > 0.1$, † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

range (Wilks' lambda = 0.63, $F_{6,118} = 11.36$, $P < 0.001$) and a marginally significant interaction (Wilks' lambda = 0.80, $F_{18,334.24} = 1.57$, $P < 0.1$, Table 1). Contrasts examining differences between the ranges in each treatment found significant effects for all treatments except for the nutrient stress. The standardized canonical correlation coefficients indicated that plants from the introduced range had larger stems (1.74), a surrogate for vegetative biomass, greater reproductive biomass (0.76), survivorship (0.30) and probability of flowering (0.47), but had fewer branches (−0.63) and were slightly shorter (−0.27). We then performed a discriminate functions analysis using population means for each treatment for the same dependent variables (Table S1). When applying cross-validation, three native populations, AA10, MNON and OTON, were consistently misclassified for all four treatments, whereas no introduced populations were misclassified in all treatments. This demonstrates that for the growth and reproductive traits measured near the end of the growing season, both Ontario populations and one population from South Dakota were more similar to the larger more fecund European plants.

We provide all univariate analyses for time-points three, four and five in the supplementary results (Tables S2 and S3). The measurements taken at the end of the experiment (T_5) reflected the general pattern found when examining these traits earlier in the season (T_3, T_4). For the final time-point (T_5), with the exception of height and branch number, introduced populations had larger vegetative traits compared with native populations. A significant influence of treatment was observed for all traits that were measured. Finally, a significant interaction was found for all traits except for plant height and branch number. Such an interaction could indicate the presence of a trade-off. However, introduced plants were generally larger than the native plants, but the degree of difference depended on the treatment and trait. The introduced populations were wider in all treatments, but this difference was only significant in the light stress (Fig. 3a). In addition, the introduced populations had significantly longer leaves in the control and light stress treatments (Fig. 3b). Similarly, the introduced plants had significantly larger stems in the control and light stress treatments (Fig. 3c).

Final above-ground biomass exhibited the same pattern as many of the size-related traits that we measured (Fig. 4a, Table 2). Introduced plants had significantly greater above-ground biomass than native plants, and there was a significant influence of treatment as well. However, there was also a significant treatment by range interaction and introduced plants produced significantly greater biomass than native plants in the control and light treatments, but not the nutrient stress treatment. Similarly, flowering introduced plants produced greater reproductive biomass compared with native plants, although the effect was only marginally significant for female biomass (Fig. 4b, Table 2). The treatments contributed significantly to the variance in

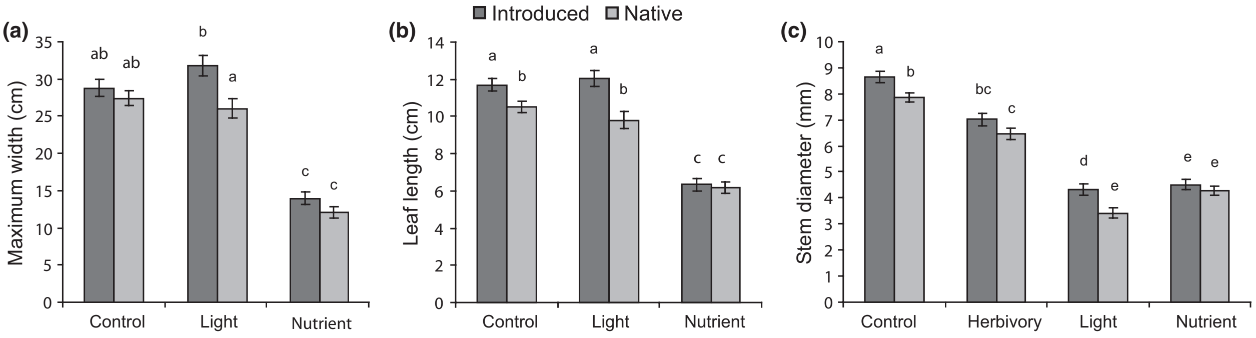

Fig. 3 Traits measured at the end of the experiment on native and introduced common ragweed (*Ambrosia artemisiifolia*) grown in four common gardens (nutrient stress, herbivory stress, light stress and control). To compare the differences in these traits between introduced and native populations, we performed a mixed model analysis. (a) Maximum width. (b) Maximum leaf length. (c) Stem diameter at the base of the plant. Least squares means and standard errors are shown. Bars with different letters are significantly different.

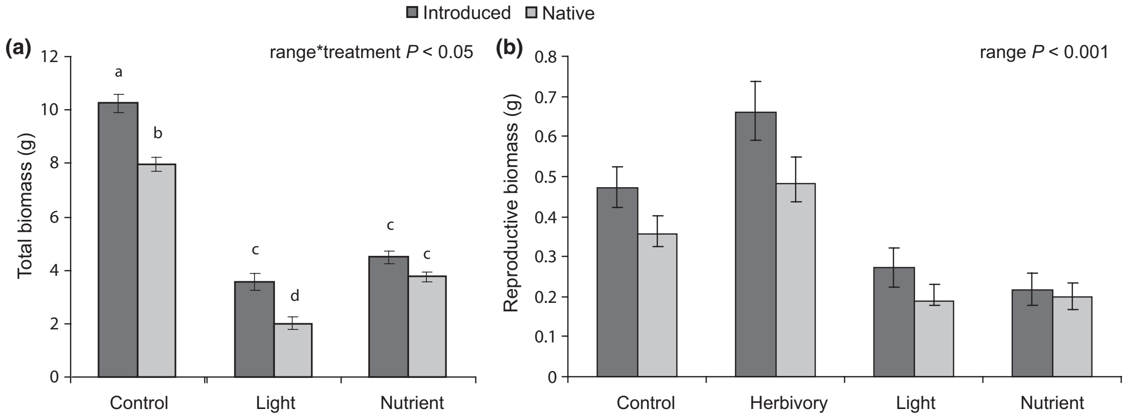

Fig. 4 Total above-ground biomass (a), and total reproductive biomass (b), measured for native and introduced common ragweed (*Ambrosia artemisiifolia*) grown in four common gardens (nutrient stress, herbivory stress, light stress and control). We performed a mixed model analysis, and least squares means and standard errors are shown. Bars with different letters are significantly different. Range, treatment and their interaction were significant for above-ground biomass. Range and treatment were significant for reproductive biomass (Table 2).

both male and female reproductive biomass, as well as the combined reproductive biomass. However, unlike the vegetative traits, there was no significant interaction ($\alpha = 0.10$) for any reproductive trait. Survivorship from the time of transplant to the end of the experiment (Fig. 5a), as well as the probability of flowering (Fig. 5b), differed between the ranges as well (Table 3). In particular, survivorship to the end of the experiment was greater in populations from Europe and a greater proportion flowered from these populations. There was no effect of treatment on survivorship, but there was a significant effect of treatment on the proportion that flowered. There was no significant interaction between range and treatment for either trait.

In order to isolate the effect of the stress on plant growth, we reanalysed the final measurements using leaf number taken prior to the start of the experiment as a covariate in an analysis of population means. We used initial leaf number as a covariate rather than initial height, as this measure was significantly different between the native and introduced populations and, unlike height, it was significantly correlated with final above-ground biomass (control treatment: leaf number $R^2 = 0.20$, $F_{1,31} = 7.70$, $P < 0.01$; height $R^2 = 0.02$, $F_{1,31} = 0.72$, $P = 0.40$). MANCOVA for the final measurements (T_5), including leaf number as a covariate, provided the same pattern of significance as the above MANOVA. Treatment, range and their interaction were significant, as was the covariate leaf number (Table 1). Interactions with the covariate were not significant ($P > 0.10$) and removed.

Univariate analysis incorporating initial leaf number revealed that this trait was correlated with all of the traits that we measured ($P < 0.05$) with the exception of height. Even when we accounted for initial size differences, greater plant size was found for introduced

Table 2 Total above-ground biomass and reproductive biomass of flowering individuals (male, female and total) measured for native and introduced common ragweed (*Ambrosia artemisiifolia*) grown in four common gardens (nutrient stress, herbivory stress, light stress and control). We performed a mixed model analysis. Treatment, range and their interaction were considered fixed effects. Block, population nested within range and maternal family nested within block, population and range as well as all possible significant interactions were considered random effects.

Fixed/random effect	Above-ground biomass	Female biomass	Male biomass	Total reproductive biomass
Treatment	$F_{2,304}$ = 848.69***	$F_{3,691}$ = 30.74***	$F_{3,271}$ = 91.23***	$F_{3,312}$ = 38.89***
Range	$F_{1,36.8}$ = 22.68***	$F_{1,28.9}$ = 3.87†	$F_{1,30.8}$ = 27.92***	$F_{1,32.4}$ = 15.38***
Range × treatment	$F_{2,304}$ = 7.67*	$F_{3,691}$ = 0.83 (ns)	$F_{3,271}$ = 2.34†	$F_{3,312}$ = 1.84 (ns)
Block	χ^2_1 =0.00 (ns)	χ^2_1 = 0.10	χ^2_1 = 0.00 (ns)	χ^2_1 = 0.00 (ns)
Population (range)	χ^2_1 = 36.10***	χ^2_1 = 15.81***	χ^2_1 = 1.00 (ns)	χ^2_1 = 1.00 (ns)
Family (range, population, block)	χ^2_1 = 9.72**	χ^2_1 = 1.50 (ns)	χ^2_1 = 0.30 (ns)	χ^2_1 = 0.00 (ns)

ns $P > 0.1$, † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

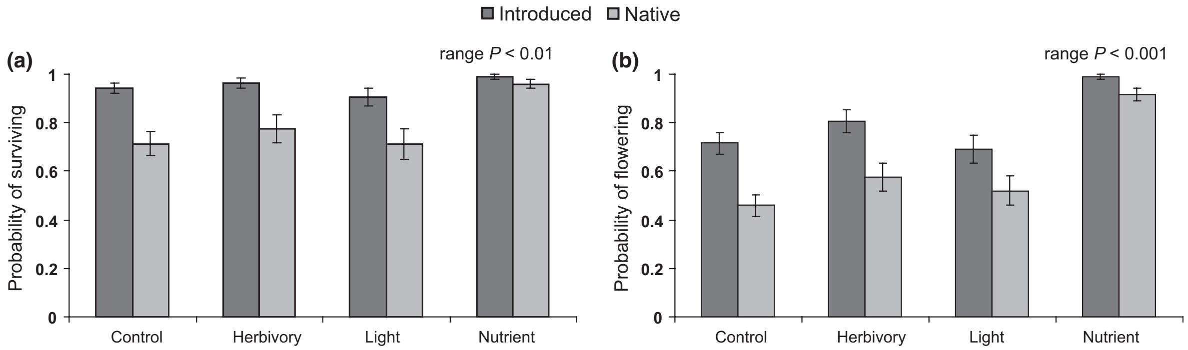

Fig. 5 The results of a generalized linear mixed model with a binary response distribution and a logit link for the probability of surviving from time-point T_1 to the end of the experiment (a) and the probability of flowering by the end of the experiment (b) for native and introduced common ragweed (*Ambrosia artemisiifolia*) grown in four common gardens (nutrient stress, herbivory stress, light stress and control). Least squares means and standard errors are shown. Refer to Table 3 for the significance of each effect in the model.

Table 3 The results of a generalized linear mixed model with a binary response distribution and a logit link for the probability of surviving from time-point T_1 to the end of the experiment, and the probability of flowering by the end of the experiment for native and introduced common ragweed (*Ambrosia artemisiifolia*) grown in four common gardens (nutrient stress, herbivory stress, light stress and control). Treatment, range and their interaction were considered fixed effects, whereas random effects were block, population nested within range and maternal family nested within population, block and range, as well as all possible significant interactions.

Fixed/random effect	Survivorship	Flowering
Treatment	$F_{3,8.977}$ = 0.52 (ns)	$F_{3,1247}$ = 13.46***
Range	$F_{1,30.45}$ = 11.06**	$F_{1,102.7}$ = 12.81***
Range × treatment	$F_{3,1247}$ = 1.45 (ns)	$F_{3,1247}$ = 0.91 (ns)
Block	χ^2_1 = 0.00 (ns)	χ^2_1 = 0.00 (ns)
Population (range)	χ^2_1 = 24.66***	χ^2_1 = 20.65***
Family (range, population, block)	χ^2_1 = 0.00 (ns)	χ^2_1 = 1.84 (ns)
Treatment × block	χ^2_1 = 18.36***	–

ns $P > 0.1$, † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

compared with native populations for width, leaf length, stem diameter and vegetative biomass, but not height or branch number (Table S4). A significant influence of treatment was observed for all traits that were measured. Finally, a significant interaction was found for vegetative biomass, width and leaf length. The introduced populations were wider and had longer leaves in all treatments, but this difference was only significant in the light stress, but not the nutrient stress or the control treatment. Vegetative biomass was significantly different in all treatments except the nutrient stress.

When we controlled for initial leaf number, we found a similar pattern of significance for total reproductive biomass (Table S4). Range, treatment as well as the interaction between treatment and initial leaf number were significant. However, the interaction between range and treatment was not significant, as introduced populations had greater reproductive biomass in all treatments (mean ± SE: introduced = 0.79 ± 0.06 g; native = 0.50 ± 0.04 g). Also, initial leaf number did not explain a significant proportion of the variation in total reproductive biomass ($F_{1,120} = 1.15$, $P = 0.27$). Finally, female reproductive biomass was a strong predictor of female flower and seed number in all treatments (female reproductive biomass $F_{1,101} = 539.85$, $P < 0.001$; treatment $F_{3,101} = 6.34$, $P < 0.001$), and the interaction between treatment and female reproductive biomass was not significant.

For survivorship and the proportion flowering, once we controlled for initial size differences, we found that range and treatment were still significant, but the interaction was not (Table S5). Therefore, introduced plants had a greater probability of surviving and producing flowers across all stresses compared with native plants even when we accounted for initial size differences.

The effects of latitude

As latitude is known to strongly influence flowering time, we examined the variation in this trait using population means and latitude as a covariate. ANCOVA revealed a negative correlation between days to first flower and latitude (Fig. 6, Table 4; slope ± SE = −2.81 ± 0.53). Overall, introduced populations flowered later than native populations (mean ± SE: introduced = 120.42 days ±1.83; native = 116.17 days ±1.41), although the range effect was not significant when we examined the number of days to the first male flower. There was no range by treatment interaction, indicating that introduced and native populations showed parallel latitudinal clines for flowering time. We also found a significant treatment × latitude interaction for days to the first male flower largely due to the effect of the light treatment, as populations produced male flowers much later than the other treatments, irrespective of the latitude (light stress slope ± SE = 0.20 ± 1.10, $t_{101} = 0.19$, $P = 0.85$).

MANCOVA of the final reproductive and growth measurements revealed a significant treatment, range and interaction between treatment and range, as well as a significant association with latitude (Table 1). Proportion flowering and total reproductive biomass most strongly influenced the canonical variate for latitude (standardized canonical coefficients: proportion flowering = 1.18, reproductive biomass = 0.57). Univariate analysis revealed that latitudinal variation was only significant for one of the size-related measurements. Stem diameter taken at 2 weeks after the start of the stress was significantly correlated with latitude ($F_{1,123} = 8.81$, $P < 0.01$), but the pattern of significance was not changed (treatment: $F_{3,123} = 11.65$, $P < 0.001$; range: $F_{1,123} = 71.49$, $P < 0.001$; treatment × range: $F_{3,123} = 3.13$, $P < 0.05$). Female biomass and reproductive biomass were positively correlated with latitude (female biomass: treatment $F_{3,123} = 12.19$, $P < 0.001$; range $F_{1,123} = 15.70$, $P < 0.001$; treatment × range $F_{3,123} = 1.82$, $P = 0.14$; latitude $F_{1,123} = 15.13$, $P < 0.001$, slope ± SE = 0.035 ± 0.008; total reproductive biomass: treatment $F_{3,123} = 28.47$, $P < 0.001$; range $F_{1,123} = 34.27$, $P < 0.001$; treatment × range $F_{3,123} = 3.78$, $P < 0.05$; latitude $F_{1,123} = 14.78$, $P < 0.001$, slope ± SE = 0.049 ± 0.012). Therefore, the pattern of significance was not changed for total female reproductive biomass, but the inclusion of latitude did alter the results for total reproductive biomass. Multiple comparisons revealed that the control, herbivory stress and light stress but not nutrient stress had greater total reproductive biomass in the introduced range. The proportion flowered was also positively correlated with latitude, but the inclusion of latitude as a covariate did not change the pattern of significance (treatment $\chi^2_3 = 77.96$, $P < 0.001$; range $\chi^2_1 = 19.95$, $P < 0.001$; treatment × range $\chi^2_3 = 2.18$, $P = 0.53$; latitude $\chi^2_1 = 16.80$, $P < 0.001$). Latitude was not significant in the population-level analysis of any

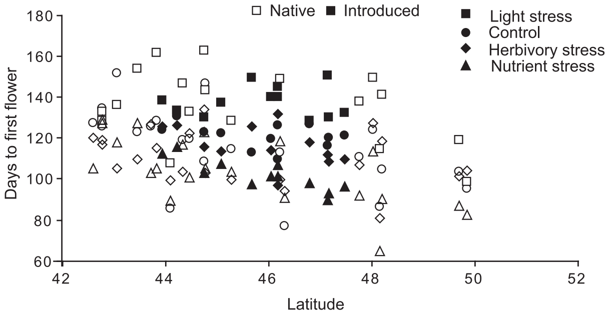

Fig. 6 Latitudinal variation in flowering time for native and introduced populations of common ragweed (*Ambrosia artemisiifolia*) grown in four common gardens (nutrient stress, herbivory stress, light stress and control). We used general linear models for an analysis on population means of the number of days until first flowering. Open symbols denote native populations, and closed symbols indicate introduced populations.

Table 4 Latitudinal variation in flowering time for native and introduced populations of common ragweed (*Ambrosia artemisiifolia)* grown in four common gardens (nutrient stress, herbivory stress, light stress and control). We used general linear models for an analysis on population means of the number of days until flowering (female, male or first flower of either sex). The fixed effects included in the model were latitude, treatment, range and treatment × range, as well as all other significant interactions.

Fixed effect	Female day	Male day	First day
Treatment	$F_{1,118} = 4.11$*	$F_{1,110} = 6.47$*	$F_{1,120} = 3.37$†
Range	$F_{3,118} = 20.36$***	$F_{3,110} = 2.03$ (ns)	$F_{3,120} = 37.58$***
Range × treatment	$F_{3,118} = 0.20$ (ns)	$F_{3,110} = 1.05$ (ns)	$F_{3,120} = 0.84$ (ns)
Latitude	$F_{1,123} = 20.27$***	$F_{1,110} = 21.77$***	$F_{1,120} = 27.24$***
Latitude × treatment	–	$F_{3,110} = 2.87$*	–

ns $P > 0.1$, † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

other trait, including germination rate and seed mass (results not presented).

Bioclimatic variation

The analysis of the bioclimatic variables yielded a $PC1_{bio}$ that explained 59% of the data, whereas the $PC2_{bio}$ explained an additional 18%. $PC1_{bio}$ was most strongly negatively correlated with temperature annual range, temperature seasonality and precipitation seasonality (eigenvector −0.291, −0.283 and −0.277, respectively) and positively correlated with precipitation in the coldest quarter and precipitation in the driest quarter (eigenvector 0.284 and 0.276, respectively). All bioclimatic variables were significantly correlated with the $PC1_{bio}$ ($P < 0.05$) with the exception of precipitation of the wettest quarter and precipitation of the wettest month (eigenvectors 0.048 and 0.047, respectively). $PC2_{bio}$ was most strongly negatively correlated with precipitation in the warmest quarter (eigenvector −0.37) and positively correlated with mean temperature of warmest quarter, maximum temperature of the warmest month and isothermality (eigenvectors 0.37, 0.34 and 0.34, respectively). Twelve of the bioclimatic variables were significantly correlated with $PC2_{bio}$. Exceptions included the five variables with the greatest eigenvectors for $PC1_{bio}$ listed earlier, as well as mean temperature of the wettest quarter and precipitation of wettest quarter. $PC1_{bio}$, but not $PC2_{bio}$, differed significantly between the ranges ($PC1_{bio}$: $t_{31} = 13.51$, $P < 0.001$; introduced mean ± SE: 4.05 ± 0.38; native mean ± SE: −2.32 ± 0.28; $PC2_{bio}$: $t_{31} = 0.70$, $P = 0.49$). This analysis demonstrates that the introduced plants, which were mainly sampled from France, generally come from populations with a wetter and more moderate climate compared with the native North American plants, which were mainly sampled from the Great Plains.

In order to determine whether the climatic differences between the sampled populations could have contributed to the observed phenotypic differences, we first performed a MANCOVA, followed by univariate ANCOVAS on population means using $PC1_{bio}$ as a covariate in the analysis. However, these results should be treated with caution, as ANCOVA is difficult to interpret when the concomitant variable ($PC1_{bio}$) is strongly correlated with another predictor variable (range), and substantial extrapolation of the regression relationships for each range may not be appropriate. Moreover, these bioclimatic variables are likely involved in the differentiation of the vegetative and reproductive traits that we observed among the populations from the native and introduced ranges and removing its effect using $PC1_{bio}$ as a covariate could lead to a misinterpretation of the results.

MANCOVA of the final measurements revealed significant effects of treatment, range, a marginally significant effect of the treatment by range interaction and $PC1_{bio}$. However, there was a significant effect of $PC1_{bio}$ × range interaction, suggesting that the relationship between the bioclimatic variables and the reproductive and growth traits differed in the two ranges (Table 1). ANCOVA revealed that $PC1_{bio}$ had a much stronger association with size-related traits compared with latitude (Table S6), although the inclusion of $PC1_{bio}$ did not change the pattern of significance in many cases. Branch number was significantly associated with $PC1_{bio}$, but as before, no effect of range was found. Final plant width and leaf length (T_5) were also significantly positively associated with $PC1_{bio}$. However, despite a significant range × treatment interaction in both cases, no significant difference between ranges for any treatment was observed. Therefore, the inclusion of $PC1_{bio}$ in the analysis removed the significant differences we previously observed for these traits in some treatments (particularly the light stress). Reproductive biomass was also associated with $PC1_{bio}$ (treatment $F_{3,123} = 27.03$, $P < 0.001$; range $F_{1,123} = 0.03$, $P = 0.86$; treatment × range $F_{3,123} = 3.59$ $P < 0.05$; $PC1_{bio}$ $F_{1,123} = 7.79$, $P < 0.01$; slope ± SE = 0.06 ± 0.02). The inclusion of $PC1_{bio}$ in the analysis removed the significant difference in reproductive biomass between the ranges, and although there was a significant treatment × range interaction, after we controlled for multiple comparisons, we found no significant differences between introduced and native plants for reproductive biomass in any of the four treatments.

As with the MANCOVA, in several cases, we also found a significant $PC1_{bio}$ × range interaction, indicating differences in the slope of the linear relationship between $PC1_{bio}$ and the traits in the two ranges. This makes the results of the ANCOVA difficult to interpret as differences between the ranges will change depending on the values of the covariate, and significant range effects only indicate significantly different y-intercepts. For all three measurements of stem diameter, there was a significant $PC1_{bio}$ × range interaction (e.g. Table S6). The slope of the relationship between the bioclimatic variable and final stem diameter was reversed in the two regions (introduced slope = −0.23 ± 0.10, $t = -2.13$, $P < 0.05$; native slope = 0.19 ± 0.057, $t = 3.35$, $P < 0.01$). Similarly, leaf length and width (time-point T_3) also exhibited a significant range × $PC1_{bio}$ interaction (Table S6). Again, the slope of the relationships between the covariate and the traits was opposite in sign and only significantly different from zero in the native range (width: introduced slope = −0.82 ± 0.49, $t_{91} = -1.65$, $P = 0.10$; native slope = 0.74 ± 0.26, $t_{91} = 2.82$, $P < 0.01$; leaf length: introduced slope = −0.39 ± 0.26, $t_{91} = -1.47$, $P = 0.15$; native slope = 0.34 ± 0.14, $t_{91} = 2.48$, $P < 0.05$). Total above-ground biomass also had a significant $PC1_{bio}$ × treatment interaction (treatment $F_{2,91} = 314.44$, $P < 0.001$; range $F_{1,91} = 2.03$, $P = 0.16$; treatment × range $F_{2,91} = 4.63$ $P < 0.05$; $PC1_{bio}$ $F_{1,91} = 3.37$, $P = 0.06$; $PC1_{bio}$ × range $F_{1,91} = 15.19$, $P < 0.001$). As mentioned earlier, the slope of the relationship between the climatic variable and biomass was opposite in sign and only significant in the native range (introduced slope = −0.20 ± 0.17, t_{91} = −1.17, P = 0.25; native slope = 0.57 ± 0.092, t_{91} = 6.12, P < 0.001).

The analysis of survivorship as well as the proportion flowering also revealed a significant PC1$_{\text{bio}}$ × treatment interaction (Table S5b). The slopes of the relationship between the two ranges were different in sign and significantly different from zero in all cases except for the probability of flowering in the introduced range (survivorship: introduced slope = −0.37 ± 0.23, χ^2_1 = −2.58, P = 0.05; native slope=0.19 ± 0.08, χ^2_1 = 5.29, P < 0.05; flowering: introduced slope = −0.17 ± 0.11, χ^2_1 = −2.57, P = 0.11; native slope = 0.17 ± 0.06, χ^2_1 = 7.59, P < 0.01). PC1$_{\text{bio}}$ was not significant in the population analysis of any other trait, including germination rate and seed mass (results not presented).

Drought stress

We found that introduced plants wilted and died significantly faster during drought stress (Fig. 7, wilted χ^2_1 = 5.90, P < 0.05; mortality χ^2_1 = 10.60, P < 0.01). These data suggest that introduced populations have reduced drought tolerance. When width was incorporated into the analysis, the significance of the range effect was removed (wilted: range χ^2_1 = 0.72, P = 0.40, width χ^2_1 = 7.23, P < 0.01; mortality: range χ^2_1 = 2.21, P = 0.14, width χ^2_1 = 10.17, P < 0.01). In contrast, height was also associated but did not remove the significance of the range effect (wilted: range χ^2_1 = 5.42, P < 0.05, height χ^2_1 = 4.97, P < 0.01; mortality: range χ^2_1 = 8.86, P < 0.01, height χ^2_1 = 7.92, P < 0.01).

Maternal effects

We found no evidence for strong maternal effects in our maternal effects experiment. As with our first experiment, we found no significant range effect for early height measurements for the maternal or offspring generation, as range and range × generation were not significant at α = 0.05 (Tables 5 and 6). However, range was marginally significant for height measured after 4 weeks (P < 0.10), with native plants growing slightly taller than the introduced plants in both generations. Range was significantly different for leaf number and width number (Tables 5 and 6), whereas range × generation was not significant, suggesting similar patterns between the ranges in both generations, where the introduced plants had more leaves and were wider than native plants.

Discussion

Our study demonstrated greater growth and reproduction of introduced common ragweed from Europe compared with native North American populations, particularly in benign environments or those that mimic above-ground competition. In contrast, the growth advantage of introduced populations resulted in poor survivorship under drought conditions. This finding is consistent with a possible trade-off between competitive ability and drought tolerance, suggesting that introduced populations may have reduced tolerance to drought as they evolved a life-history strategy that favours more rapid growth and reproduction. The apparent greater drought tolerance of native populations is likely related

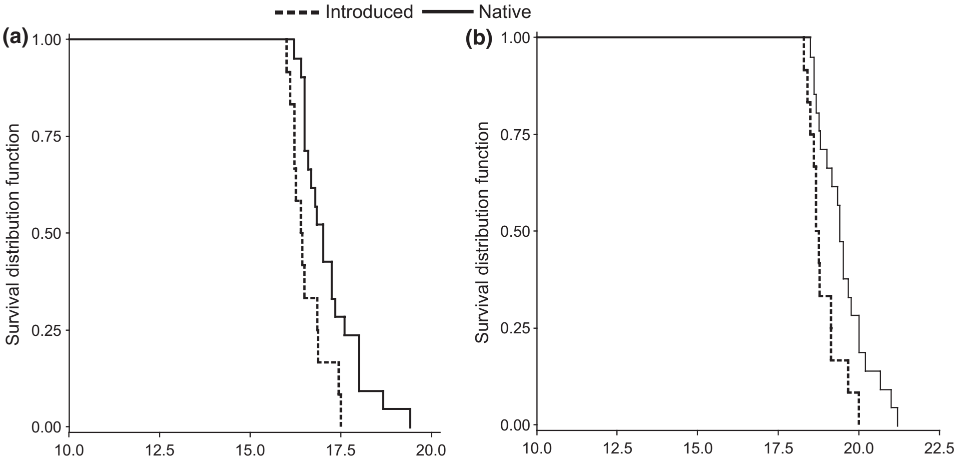

Fig. 7 The effects of drought on wilting and survivorship (population medians for days until wilting and days until death from the cessation of watering) for 12 native and 22 introduced populations of common ragweed (*Ambrosia artemisiifolia*) grown in a common garden. (a) The frequency of populations where half of the individuals had not yet wilted at each time-point (range χ^2_1 = 5.90, P < 0.05). (b) The frequency of populations where half of the individual had not yet died at each time-point (range χ^2_1 = 10.60, P < 0.01). We found that introduced plants wilted and died significantly faster during drought stress.

Table 5 Height, leaf number and width for native and introduced common ragweed (*Ambrosia artemisiifolia*) measured 2 weeks (T_1) and 4 weeks (T_2) after germination for maternal plants and their offspring grown in a common garden. We performed a mixed model analysis. Generation, range and their interaction were considered fixed effects. Population nested within range and maternal family nested within population and range, as well as all possible significant interactions, were considered random effects.

Fixed/random effect	Height (T_1)	Leaf number (T_1)	Height (T_2)	Width (T_2)
Range	$F_{1,8.6} = 0.01$ (ns)	$F_{1,11.32} = 5.19^{*}$	$F_{1,13.8} = 4.24^{\dagger}$	$F_{1,17.15} = 58.03^{***}$
Generation	$F_{1,227.5} = 33.2^{***}$	$F_{1,230.8} = 7.66^{***}$	$F_{1,221.9} = 37.39^{***}$	$F_{1,218.55} = 97.53^{***}$
Range × generation	$F_{1,227.5} = 0.36$ (ns)	$F_{1,230.8} = 3.44^{\dagger}$	$F_{1,221.9} = 0.59$ (ns)	$F_{1,218.55} = 0.13$ (ns)
Population (range)	$\chi_1{}^2 = 1.28$ (ns)	$\chi_1{}^2 = 1.43$ (ns)	$\chi_1{}^2 = 0.00$ (ns)	$\chi_1{}^2 = 1.70$ (ns)
Family (range population)	$\chi_1{}^2 = 12.31^{***}$	$\chi_1{}^2 = 1.22$ (ns)	$\chi_1{}^2 = 1.20$ (ns)	$\chi_1{}^2 = 17.95^{***}$

ns $P > 0.1$, † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 6 Height, leaf number and width for native and introduced common ragweed (*Ambrosia artemisiifolia*) measured 2 weeks (T_1) and 4 weeks (T_2) after germination for maternal plants and their offspring grown in a common garden. Least squares means from the analysis and standard errors are shown. Trait means that are significantly different between the ranges are in boldface.

Trait	Maternal introduced	Maternal native	Offspring introduced	Offspring native
Height T_1 (mm)	73.6 ± 4.8	72.1 ± 5.4	52.9 ± 3.3	55.3 ± 3.9
Leaf number	**3.4 ± 0.3**	**3.1 ± 0.3**	**4.4 ± 0.2**	**3.3 ± 0.2**
Height T_2 (cm)	10.3 ± 0.5	11.6 ± 0.6	8.1 ± 0.3	8.7 ± 0.3
Width T_2 (cm)	**21.4 ± 0.8**	**15.9 ± 0.9**	**14.6 ± 0.4**	**9.6 ± 0.5**

to the bioclimatic differences between the native and the introduced regions that we sampled. We also found parallel latitudinal clines for flowering time and reproductive biomass, providing evidence for rapid adaptation to local environmental conditions during the invasion of Europe by common ragweed.

Our data provide evidence for genetic differentiation between European and North American populations for several traits, including plant width, vegetative and reproductive biomass, survivorship and flowering time (e.g. Table 1). We found improved seedling growth in introduced European populations, and this advantage extended throughout the growing season to the end of the experiment and was apparent regardless of the stress treatment that we applied, although growth under drought stress was not measured. This advantage does not appear to be due to maternal effects, as F_1s, produced from intrapopulation crosses of glasshouse-reared parental plants, maintained this growth difference between the ranges (Tables 5 and 6). Similarly, survival and reproduction were greater in the introduced range for all treatments with the exception of drought. The evolution of increased growth and reproduction in alien plant populations has been observed in several invasive species (e.g. Blair & Wolfe, 2004; McKenney *et al.*, 2007; He *et al.*, 2010; reviewed in Bossdorf *et al.*, 2005), although many exceptions have also been identified (e.g. Willis *et al.*, 2000; van Kleunen & Schmid, 2003).

In our experiment, increased growth and reproduction in the light stress environment, which mimicked aspects of above-ground shading by other plants, indicated a substantial advantage of the introduced populations over native ones, suggesting adaptation to more competitive environments in European populations. Specifically, our light treatment exposed common ragweed to reduced quality and quantity of light and individuals, particularly European plants, responded by growing taller, and producing larger leaves relative to their biomass (e.g. Figs 3 and 4), a common response to shading in many plant species (Fitter & Hay, 1987; Sultan & Bazzaz, 1993; Poorter & Nagel, 2000). By increasing the amount of photon-harvesting surface, greater leaf area can be a key trait involved in maintaining growth rate under low light levels (Poorter & Nagel, 2000; Steinger *et al.*, 2003). This improved shade avoidance response of the introduced common ragweed populations likely functioned to increase their survival and reproductive success in the light stress relative to the native populations (Figs 4 and 5).

The evolution of higher growth rates is a classic response to competitive environments (Grime, 1977; Chapin *et al.*, 1993). For example, artificial selection on a ruderal mustard under intense competition resulted in the evolution of more rapid growth (Miller, 1995). In contrast, slower growth can evolve in response to a resource-poor environment, due to common physiological mechanisms underlying growth and stress responses, so that heritable changes in growth rates have negative pleiotropic responses to stress (Grime, 1977; Chapin *et al.*, 1993). Consequently, trade-offs are expected between allocation to growth and reproduction and abiotic stress tolerance. Empirical evidence for such trade-offs is numerous and includes metal-tolerant populations and species distributed along resource gradients (e.g. Antonovics *et al.*, 1971; Jurjavcic *et al.*, 2002; Petrů *et al.*, 2006; Maestri *et al.*, 2010). Interestingly, in our experiment, higher growth and reproduction of the introduced populations was maintained across four different environments, although the difference was lessened in the nutrient stress, suggesting greater tolerance to a wider array of environmental conditions compared with native populations. Such improved resistance and tolerance to stress could aid invasion, as a species is likely to encounter variable environmental conditions during extensive range expansion (Baker & Stebbins, 1965; Bazzaz, 1986; Alpert *et al.*, 2000; Richards *et al.*, 2006; Davidson *et al.*, 2011).

We detected more rapid mortality of the introduced common ragweed under drought conditions (Fig. 7). This pattern was largely explained by the increased size of the European plants, which have relatively more water-consuming tissue and larger leaf surface area from which to lose water. It is unclear whether the North American ragweed populations have other morphological, physiological and biochemical adaptations to water stress that have been frequently found in other plant species (Heschel *et al.*, 2002; Xiong & Zhu, 2002). Indeed, we cannot rule out the possibility that the higher above-ground growth rate observed in introduced ragweeds is accompanied by greater root growth, which might compensate for higher rates of water loss in the field. If so, the trade-off we observed in the greenhouse might not be seen under more natural conditions. Moreover, further investigation into the existence of a negative genetic correlation between growth rate and drought tolerance in this species is warranted to demonstrate a genetically based trade-off. However, previous studies have found that plants with relatively low growth rates survive for longer time periods during drought (Givnish, 1979; Donovan & Ehleringer, 1992; Ehleringer, 1993). In addition, several interspecific studies have shown invaders to be less drought tolerant than closely related native species, perhaps owing to their greater growth rate (Brock & Galen, 2005; Castillo *et al.*, 2006; Garcia-Serrano *et al.*, 2009), although the evolution of reduced drought tolerance in weedy and invasive populations has received little attention in the literature (but see He *et al.*, 2010).

We clearly demonstrated genetic differences between the sampled North American and European populations, which likely reflect adaptation to differing biotic and climatic conditions. However, a reciprocal transplant experiment involving North American populations and a French population of common ragweed did not find evidence of increased growth in the introduced range (Genton *et al.*, 2005b). Our contrasting findings are likely because the earlier study investigated only three populations (one from North Carolina, one from Ontario and one from France), whereas our study encompassed a much larger survey of populations in the native and introduced range. The local environmental conditions of the sampled native and introduced populations from each study likely played an important role in determining the observed degree of difference between the ranges. The majority of our samples were from the Great Plains, from which the present-day North American populations may have originated. This region has more extreme seasonality, is drier and has a shorter growing season than the North American populations from the Genton *et al.*'s (2005b) study. Interestingly, one Ontario population from our study (MNON, Table S1) was consistently misclassified based on the morphological and life-history traits as introduced using a discriminant functions analysis, as it exhibited more rapid growth and greater reproduction compared with other North American populations. This could be a response to the more moderate, European-like climatic conditions in Southern Ontario. Moreover, common ragweed is problematic in this region and one of the most abundant weeds of agricultural fields (Alex & Switzer, 1992). The phenotypic similarity is unlikely solely due to invasion history as genetic evidence suggests that common ragweed in Europe (including France) is sourced from many regions of North America (Genton *et al.*, 2005a; Chun *et al.*, 2010, 2011; Gaudeul *et al.*, 2011).

The evidence for the evolution of increased competitive ability in response to enemy release (Blossey & Notzold, 1995) has been found in numerous plant invaders (Bossdorf *et al.*, 2005), including other composite weeds (Caño *et al.*, 2009). Although we found greater growth and reproduction in European populations, we found no evidence for greater tolerance to herbivory in the native range, which may be one possible response to reduced herbivory loads in the introduced range (van Kleunen & Schmid, 2003; Müller-Schärer *et al.*, 2004; Bossdorf *et al.*, 2005). In fact, we found the opposite pattern, with greater tolerance to simulated herbivory by the introduced populations, a result consistent with some other invasive plants (Huang *et al.*, 2010), perhaps owing to their more rapid growth. In addition, a preliminary experiment examining the preference of the generalist herbivore *Trichoplusia ni* for native or introduced plants provided no evidence for differential resistance to herbivory, as native and introduced leaf discs were chosen with equal frequency by the herbivore (results not presented). A previous survey of the populations in eastern North America and France found lower levels of damage in the French populations, suggesting that enemy release could be an important factor in the success of common ragweed in Europe (Genton *et al.*, 2005b). However, they found no evidence for an evolutionary response to differing herbivory loads, as reciprocal transplant in the same study found equivalent levels of damage in both European and North American populations.

Similar to our findings, evidence for reduced investment in defence has been found to be lacking in several studies of invasive populations (for review, see Bossdorf *et al.*, 2005). Selection by generalist insect herbivores might be sufficient to maintain high tolerance and resistance in the introduced range. Alternatively, it is possible that other agents of selection in the introduced range have maintained herbivory tolerance (e.g. mowing or drought), as similar mechanisms may underlie the response to these factors (e.g. Strauss & Agrawal, 1999; van Kleunen & Schmid, 2003; Müller-Schärer *et al.*, 2004). Such explanations may underlie why we and others have found no evidence for reduced resistance or tolerance to herbivores in introduced populations of common ragweed. We did not examine resistance to specialist herbivores, and it is possible that reduced investment in defence traits specific to specialists occurs in the introduced range, which has been found in other invasive species (Joshi & Vrieling, 2005; Huang *et al.*, 2010), as few specialist herbivores are found on common ragweed in Europe (Genton *et al.*, 2005b).

The demographic history of introduced populations can have important consequences for the evolution of invading populations. Founder effects and population bottlenecks that invaders experience can potentially play a large role in the degree of phenotypic and genetic differentiation between continents (Bossdorf *et al.*, 2005; Dlugosch & Parker, 2008a). High genetic variation and admixture of common ragweed populations in Europe (Genton *et al.*, 2005a; Chun *et al.*, 2010, 2011; Gaudeul *et al.*, 2011; Gladieux *et al.*, 2011) suggest that founder effects and populations bottlenecks do not play a large role in the genetic differences that we observed. However, future work investigating the relationship between European populations and the portions of the North American range studied here is warranted.

Flowering phenology and reproductive biomass diverged among populations resulting in latitudinal clines, suggesting adaptation to local climatic conditions with both the North American and European ranges of common ragweed (Fig. 6 and Table 4). Similar to our findings, Chun *et al.* (2011) found latitudinal clines for flowering time in French common ragweed populations. Observations of parallel clines in life-history traits within the native and introduced ranges have demonstrated that introduced species can evolve rapidly in response to abiotic gradients (reviewed in Colautti *et al.*, 2009). Human-mediated movement of genotypes between climatically similar regions could essentially transplant clinal patterns of variation found in the native range to the introduced range (Maron *et al.*, 2004; Keller & Taylor, 2008). Such climate matching seems unlikely in common ragweed given the level of admixture in Europe (Genton *et al.*, 2005a; Chun *et al.*, 2010, 2011; Gaudeul *et al.*, 2011). We observed slightly later flowering times for the same latitudes in Europe, which could reflect adaptation to a milder climate and longer growing season in Europe compared with the North American populations. Weedy or ruderal species are thought to respond to abiotic stress through earlier flowering times (Stanton *et al.*, 2000), and the bioclimatic data indicate that the sampled populations of the native range may experience harsher conditions, potentially contributing to the flowering time differences between the ranges.

We also observed parallel clines in allocation to female function, as populations in both the native and introduced range had greater female reproductive biomass at higher latitudes. This pattern was also observed in a recent study examining differentiation in flowering time and reproductive allocation among common ragweed populations in France (Chun *et al.*, 2011). The higher reproductive output of northern populations could reflect the earlier flowering times of these populations and their greater opportunity to reproduce in the glasshouse. Interestingly, we did not observe corresponding latitudinal clines in other traits, such as vegetative biomass, which would be predicted under life-history theory due to trade-offs between body size and age of maturation (Roff, 1992) and has been observed in other invasive plants (e.g. Colautti *et al.*, 2010). Variation among populations in these size-related traits was generally correlated with bioclimatic variables, but not latitude.

We found parallel responses to bioclimatic variation in the native and introduced ranges for several traits, including reproductive biomass and plant width. However, in several cases, we found significant interactions between climate and range, where the slopes of relationship between $PC1_{bio}$ and the traits were different in sign, suggesting that climatic variables are affecting ragweed populations differently in the two ranges. Specifically, in the native range, populations from wetter and more moderate climates grew larger and had higher survivorship and a greater probability of flowering, whereas this pattern was reversed in the introduced range. This difference could be due to several factors, such as a nonlinear relationship between climate of origin and growth and survival common to both ranges, as $PC1_{bio}$ was largely nonoverlapping for the two types of populations. Alternatively, differences in other abiotic and biotic factors between the two ranges or the effects of invasion history on patterns of population differentiation could be responsible for these differences.

Theory reveals that local adaptation to differing biotic and abiotic environments resulting in improved growth and reproduction of introduced populations can contribute to population expansion (Hastings, 1996; Holt *et al.*, 2005). This suggests that it is not the evolution of higher growth rates *per se* that is important for invasion success, but adaptation to local environmental conditions in the new range that promotes population growth and invasion. The differences that we observed between the native and introduced range of common ragweed and their association with latitude and climate suggest that there has been adaptation to local environmental conditions during invasion. Future reciprocal transplant experiments and studies of selection are required to confirm this finding.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 A list of population locations for the common ragweed populations used in this study.

Table S2 Maximum height, width, leaf length and stem diameter at time-point three a), four b), and five, c), (T3, T4 and T5) for native and introduced common ragweed grown in four common gardens.

Table S3 Traits measured at time-point three, four and five (T3, T4 and T5) on native and introduced common ragweed grown in four common gardens.

Table S4 Results of an ANCOVA using population means of traits measured at the end of the experiment time-point five (T5) the on native and introduced common ragweed grown in four common gardens.

Table S5 The results of a generalized linear model with a binomial response distribution and a logit link for the proportion that survived in each population from time-point T1 to the end of the experiment, and the proportion in each population that flowered by the end of the experiment for native and introduced common ragweed grown in four common gardens.

Table S6 Results of an ANCOVA using population means of morphological traits measured the on native and introduced common ragweed.

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