Rapid genomic and phenotypic change in response to climate warming in a widespread plant invader

Yan Sun | Oliver Bossdorf | Ramon D. Grados | ZhiYong Liao | Heinz Müller-Schärer

Abstract
Predicting plant distributions under climate change is constrained by our limited understanding of potential rapid adaptive evolution. In an experimental evolution study with the invasive common ragweed (Ambrosia artemisiifolia L.) we subjected replicated populations of the same initial genetic composition to simulated climate warming. Pooled DNA sequencing of parental and offspring populations showed that warming populations experienced greater genetic divergence from their parents, than control populations. In a common environment, offspring from warming populations showed more convergent phenotypes in seven out of nine plant traits, with later flowering and larger biomass, than plants from control populations. For both traits, we also found a significantly higher ratio of phenotypic to genetic differentiation across generations for warming than for control populations, indicating stronger response to selection under warming conditions. As a measure for evolutionary rate, the phenotypic and sequence divergence between generations were assessed using the Haldane metric. Our approach combining comparisons between generations (allochronic) and between treatments (synchronic) in an experimental evolutionary field study, and linking population genomic data with phenotyping analyses provided a powerful test to detect rapid responses to selection. Our findings demonstrate that ragweed populations can rapidly evolve in response to climate change within a single generation. Short-term evolutionary responses to climate change may aggravate the impact of some plant invaders in the future and should be considered when making predictions about future distributions and impacts of plant invaders.

KEYWORDS
Ambrosia artemisiifolia, biological invasions, climate change, common ragweed, experimental evolution, phenotypic evolution, selection experiment
1 | INTRODUCTION

There is overwhelming evidence that the global climate is changing rapidly (IPCC, 2013). Besides a rapid increase in global temperatures, the frequency of extreme weather events is also increasing, often confronting species with environmental conditions they rarely experienced in their recent evolutionary histories (Chevin & Hoffmann, 2017). Most previous research has focused on range expansion or contraction due to changes in environmental settings, while evolutionary responses to climate change are still under studied, even though it is well known that historical climate changes have often been accompanied by evolutionary changes (Alberto et al., 2013; Lavergne, Mouquet, Thuiller, & Ronce, 2010; Nadeau & Urban, 2019). In parallel to climate change, biological invasions, another important driver of ecological changes in the Anthropocene (Merow, Bois, Allen, Xie, & Silander, 2017), are also increasingly threatening native ecosystems, with no saturation yet in the accumulation of alien plants worldwide (Seebens et al., 2017) and thus with increasing costs for their management (Vilà et al., 2011). Theoretical and empirical studies indicate that climate change may exacerbate the risk of plant invasions (Sandel & Dangremont, 2012), but understanding the interactive effects of climate change and invasions remains challenging, particularly since invasive populations may evolve rapidly.

Biological invasions are often accompanied by significant demographic or evolutionary events such as genetic bottlenecks, hybridization and admixtures, and therefore invasive populations are often genetically different from populations in the native range (Alexander & Edwards, 2010; Estoup et al., 2016). Moreover, there is often intra-specific hybridization in invasive populations (Rius & Darling, 2014). Population admixtures before introductions or after multiple introductions from genetically distinct sources have repeatedly been found to overcome initial demographic bottlenecks, thereby solving the so-called “genetic paradox of invasion” (Dlugosch, Anderson, Braasch, Cang, & Gillette, 2015), the invasion success of some species despite strong demographic bottlenecks. In a recent review, Dlugosch et al. (2015) reported admixture of 37% of 70 invasive species studied with nuclear markers. Such admixture can increase genetic variance within populations, and it can result in heterosis, as well as novel or transgressive phenotypes (Bock et al., 2015; Rius & Darling, 2014). Moreover, escape from specialized natural enemies and mutualists may strongly alter biotic interactions of invasive plants in their introduced range (Strauss, 2014; Zuppinger-Dingley et al., 2011), which could relax selection on multiple traits, allowing populations to adapt more rapidly to novel environmental conditions than native species.

Microevolutionary change in plants can indeed be fast (Bone & Farres, 2001), with several examples in particular from climate-change experiments and invasive plant populations (Chown et al., 2015). Recent studies found, for instance, rapid molecular divergence in plants subjected to experimental climate change (Ravenscroft, Whitlock, & Fridley, 2015), usually with reduced genetic variation under selection (cf. review Hoffmann & Sgrò, 2011). Other studies showed rapid evolution of plant phenology in invasive *Lythrum salicaria* populations (Colautti & Barrett, 2013), and rapid geographic differentiation of flowering time (Weber & Schmid, 1998) as well as root:shoot ratio and water-use efficiency (Li, Du, Guan, Yu, & van Kleunen, 2016) among invasive *Solidago canadensis* populations in Europe and China respectively.

In order to predict future ecological consequences of plant invasions, a better understanding of the pace and extent of their evolutionary responses to climate change is crucial (Moran & Alexander, 2014). As suggested above, evolutionary responses to climatic change might be even stronger in introduced than in native populations, being further fostered by their relative isolation from gene flow from the native range, as compared with spreading native population, and we may thus expect evolutionary changes influencing range expansion, niche differentiation and ecological impact in invasive plant populations (Gallien et al., 2016; Prentis, Wilson, Dormontt, Richardson, & Lowe, 2008).

Microevolution of plant populations in response to environmental change has so far mostly been studied in two ways: First, researchers have measured heritability of target traits as well as contemporary selection on them, and then made predictions about the potential for short-term evolution of traits (Colman et al., 2003). Second, other studies compared populations or species that have diverged over time and made inferences about the processes that drove evolutionary change in the past, such as in biological invasion (van Boheemen, Atwater, & Hodgins, 2019; Chun, Le Corre, & Bretagnolle, 2011; Li, Liao, Wolfe, & Zhang, 2012). A third, and so far less used, approach often considered the ‘gold standard’ of microevolutionary studies are selection experiments—also called experimental evolution—where microevolution is observed ‘in action’ (but e.g. Agrawal, Hastings, Johnson, Maron, & Salminen, 2012). Experimental evolution studies can follow evolutionary dynamics under different selection regimes, and assess the repeatability (and thus predictability) of evolution across replicated experimental populations (Agrawal et al., 2012; Kawecki et al., 2012; Schlötterer, Koffer, Versace, Tobler, & Franssen, 2015). To improve a mechanistic understanding of evolutionary changes, de Villemereuil, Gaggiotti, Mouterde, and Till-Bottraud (2016) proposed combining population genomics with a common-garden approach. Using this approach, Barghi et al. (2019) recently combined a laboratory selection experiment to detect selection signatures with phenotyping in a common environment and showed that *Drosophila simulans* populations harbour a vast evolutionary potential for future temperature adaptation. So far, experimental evolution studies have rarely been done in natural settings (Müller-Schärer et al., 2020; Schlötterer et al., 2015).

One of the key traits associated with plant responses to environmental stress is specific leaf area (SLA), which is determined by a trade-off between cell size and cell density (Mousseau, 1999). For instance, Hudson, Henry, and Cornwell (2011) found increased SLA in a long-term artificial warming experiment with *Cassiope tetragona*. Another important trait in the context of climate change responses is relative water content (RWC), an indicator of plant water deficit and of the capacity of a plant to avoid dehydration under different growing conditions (Barrs & Weatherley, 1962; Mullan & Pietragalla, 2012). Under water deficit conditions, there is often a negative relationship between SLA and RWC, with lower SLA reflecting thicker leaves.
that can maintain a higher RWC (Maisto, Santorufo, & Arena, 2013). Net assimilation rate (NAR), a proxy for a plant’s efficiency in using CO₂ for dry matter accumulation, was found to significantly differ in *Saccharum officinarum* between control and heat stress over time and space (Wahid, 2007).

Here we studied one of the most noxious plant invaders in Europe, common ragweed *Ambrosia artemisiifolia* L. (Asteraceae; hereafter: ragweed), causing some 13.5 million people to suffer from ragweed-induced allergies in Europe, with annual economic costs of approximately 7.4 billion Euros (Schaffner et al., 2020). Species distribution models predict a northward spread of ragweed under climatic change both for the introduced European, East Asian (Chapman et al., 2016; Sun et al., 2017; Sun, Zhou, Wang, & Müller-Scharer, 2018) as well as its native North American range (Case & Stinson, 2018) and Hamauvi-Laguel et al. (2015) predicted a four times higher pollen concentrations in Europe by 2050. In general, populations may either migrate to follow suitable environmental conditions in space without evolving, or they might locally adapt to novel climatic conditions, with or without migrating (Polechová, Barton, & Marion, 2009). However, even during migration, plants may experience new abiotic and biotic conditions that could exert selection and thus also migrating populations are expected to evolve. Although all of these processes will affect plant distributions under climate change, evolutionary changes have so far been largely ignored in species distribution models (Lavergne et al., 2010).

Ragweed is a wind-pollinated and obligate out-crossing annual, with outcrossing rates of 0.93–1.0 recorded in both native and introduced populations (Friedman & Barrett, 2008; Li et al., 2012). Moreover, previous studies found high genetic variation within introduced ragweed populations in Europe (van Boheemen et al., 2017; Gerton, Shykoff, & Giraud, 2005; McGoe & Stinchcombe, 2018), most likely because of multiple introduction and pre-admixtures (Gaudeul, Giraud, Kiss, & Shykoff, 2011). This resulted in very large effective population sizes, substantial standing genetic variation in ecologically important traits and novel genetic substrate, as compared with native populations, and on which selection can act and potentially generate lineages with greater or shifted ecological amplitudes or fitness (Colautti & Lau, 2015; Rius & Darling, 2014).

**Figure 1** Experimental setup of the field experimental evolution and subsequent phenotyping study. (a) Geographic locations of the 19 North Italian populations (purple points) from which the 60 maternal families of *Ambrosia artemisiifolia* were collected and used as the genetic starting material in the field experiment (location indicated by the orange star in a). (b) Lay-out of the field experimental evolution study, with control cages (blue) and warming treatment cages (red) used in this study (the black quadrats are another treatment not part of this study), each yellow block contains one pair of the two treatments. (c) Left side: Overview of the two generations of the field experiment. Each cage population was founded by 120 individuals, with two individuals from each of the 60 maternal families (grey frames on top; parental generation). Blue and red frames indicate the populations established by the first-generation offspring seeds (bottom) for control (blue filled quadrats) and warming treatments (red filled quadrats); respectively. Right side: In the growth camber study, the grey, blue and red framed quadrats represent the parental plants (the same 60 maternal families from the 19 field populations, which were also used to start the field experiment) and offspring from the field control and warming treatment respectively. Dashed arrows indicate use of seed sources, and solid arrows are comparisons of molecular or phenotypic comparisons made between generations, with the G_C and G_W referring to the rates of sequence divergence between generations, and P_C and P_W referring to the rates of phenotypic divergence between generations (see details in Section 2)
To test the potential for rapid evolutionary response to climate change, we studied the first offspring generation from an ongoing experimental evolution study established in 2016 in which replicated populations of identical initial genetic composition had been subjected to simulated climate warming or ambient (control) climate conditions. We employed population genomic tools (pooled DNA sequencing) to test for genomic changes, and a common-environment study using seeds from all parental and offspring populations to assess evolutionary changes in phenotypes. Combining comparisons both between generations (allochronic) and between treatments (synchronic; cf. Figure 1c, Section 2) allowed to specifically ask (a) whether there were significant molecular changes across generations, and if these differed between warming and control populations; (b) whether such molecular changes were accompanied by changes in phenotypic traits; and (c) if so, whether a comparison between phenotypic and molecular changes indicated phenotypic selection and thus rapid genetic response to selection.

2 | MATERIALS AND METHODS

Common ragweed, native to North America, has become a problematic alien invasive plant in many continents, for example, Asia, Oceania and Europe (Essl et al., 2015). It causes great damage to societies because of its highly allergenic pollen (Schaffner et al., 2020), and because it is also an important and hard-to-control crop weed (Müller-Schärer et al., 2018). Because of these problems, and since it is predicted to further expand in Europe under climate change, ragweed has greatly contributed to the awareness of the invasive species problem in Europe (Müller-Schärer et al., 2018).

2.1 | Field experiment

In 2016, we set up five pairs (blocks) of cages (2 × 2 × 2 m) with genetically similar experimental ragweed populations in a field in Magnago, Northern Italy (Figure 1). The municipality of Magnago, located in one of the most infested ragweed areas of Europe, provided an ideal environment for our field selection experiments in an enclosed grassland property where ragweed had been observed only very rarely before, and therefore no ragweed was expected in the soil seed bank. Each caged population was founded by 120 individuals (=50 plants/m², corresponding to frequently observed field densities in Europe; Lommen et al., 2018), with two individuals from each of the same 60 maternal families that had previously been sampled from 19 invasive ragweed populations in 2013–2015 (2–4 maternal families per population; Figure 1) within a radius of 35 km to bolster the earlier described high genetic within population variation in Europe (van Boheemen et al., 2017; McGoey & Stinchcombe, 2018). Half of the experimental populations were subjected to simulated climate warming created by an open-top Plexiglas chamber, which increased the temperature, but minimized other ecological effects (Marion et al., 1997). All plots were covered with fine-meshed tissue to protect the plants from herbivory.

To verify our climate treatments, we installed temperature loggers in each experimental population and compared their measurements with the climate data in the predicted current and future ragweed distribution areas based on Sun et al. (2017), Sun et al. (2018) and climate data obtained from www.worldclim.org. The warming plots in our experiment showed a higher diurnal temperature range (t = 3.76, df = 1,004, p < .001), an average 2.2°C increase of daily mean temperature (t = 3.63, df = 1,004, p < .001) and an average 3°C increase of daily maximum temperature (t = 3.84, df = 1,004, p < .001) compared with the control plots during the growing season (Figure S1a–c). These changes were qualitatively similar to future climate changes predicted for the area suitable for ragweed during the growing season in Europe, with larger temperature ranges (t = 98.42, df = 85,577, p < .001), an average 3.8°C increase in monthly mean temperature (t = 1751.2, df = 85,577, p < .001) and an average 4.8°C increase in monthly maximum temperature (t = 1,332.7, df = 85,577, p < .001; Figure S1d–f).

Our experimental conditions thus mimicked the predicted future climate changes reasonably well.

2.2 | Genomic analyses

To assess molecular diversity and differentiation among experimental generations and treatments, we collected leaf tissue from all 120 parental individuals in each of the 10 caged field plots in Italy in June 2016, and then again from 120 offspring individuals in each plot in June 2017 (one random individual per cell in a 100 × 120 cm grid in the centre of the plot). Thirty caged populations each containing equal amounts of each individual plant tissue (~1 mg) were then used for DNA sequencing on an Illumina HiSeq3000 at the Max Planck Institute for Developmental Biology in Tübingen (see Appendix S1C for details on DNA extraction, library preparation and processing of the raw data).

After the pipeline processing of the sequencing data from 20 population pools, we retained 1,786,946,216 paired-end reads (97.9%), corresponding to 311.1 Gb of sequence data with a median Phred-score of 37. Duplicate filtering resulted in removing 8.5%–11.2% of mapped reads and only 2%–2.6% were considered as missing data. The remaining reads corresponded to an average 30x coverage per population pool. We estimated genetic variability within populations as π with the Perl script ‘Variance-sliding.pl’ in ‘POPOOLATION’ (Kofler, Orozco-terWengel, et al., 2011). Next, we calculated the genetic differentiation (FST) between each parental and offspring generation with ‘fst-sliding.pl’ in ‘POPOOLATION2’ (Kofler, Pandey, Pandey, & Schlötterer, 2011), using a sliding-window approach with a window size of 1,000 bp and 1,000 bp steps to identify SNPs and genomic regions with elevated differentiation between generations. We generated position-specific nucleotide metrics with ‘bam-readcount’ (version 0.8.0, https://github.com/genome/bam-readcount), and we analysed the bam-readcounts with a custom-written Perl script to detect polymorphic sites (c. 3 million SNPs were identified in each pooled population). The outputs were then used for principal component analysis (PCA) to visualize the genetic differences between the two generations for the control versus warming treatments. The results for the differentiated SNPs and their
annotations will be reported elsewhere. Differences between generations in and between treatments in $F_{ST}$ were analysed with nonparametric Kruskal–Wallis tests in R.

2.3 | Phenotype assays

To assess phenotypic divergence between the treatments, after differential mortality, growth and reproduction of the originally sown genotypes had occurred, we collected five mature seeds of each individual branch in the centre, at about 100 × 120 cm of each parental population, typically representing over a hundred (103–119) individuals. We used these seeds, together with the original seeds used to establish the parental populations, to set up a common-environment study at the University of Tübingen (see Appendix S1D for further details on growing conditions). Eventually, we were able to grow 141 plants from the parental generation (47 out of the 60 maternal families from 16 populations due to low seed/seedling numbers of some families, with three replicates each) and 282 offspring plants (25–30 individuals per population) in a climate-controlled growth chamber (see Appendix S1D for details), with positions of plants randomized every fortnight. Each plant received 60 ml tap water per day.

Prior to the experiment, we used 70 offspring seeds to calculate the relationship between seed area and seed biomass, and we later used this formula to estimate the average seed biomass of each offspring population based on the total area of 500 seeds obtained with ImageJ (version 1.51k; http://imagej.nih.gov/ij/). We recorded germination daily over 3 weeks, and considered seeds as germinated when the embryo was completely uncoiled. Three days after transplanting, we measured the initial height of each plant, and we also measured the height and biomass of 30 extra seedlings and calculated their correlation to be able to non-destructively estimate the initial biomass of all plants. Because of the highly allergic pollen of ragweed, and regional health regulations, we could not have open flowers in the growth chamber at the University of Tübingen, and this precluded growing plants first for another generation under controlled conditions to reduce maternal effects. We harvested each plant when it began to flower, that is, the male inflorescence had reached 1 cm, which was 32–90 days after transplanting. By the end of the experiment at day 90, only nine individuals had not flowered yet and were assigned as 91 days for flowering-time analyses. As ragweed plants tended to reach their maximum height and biomass at the time of flowering (Lommen et al., 2018; Sun & Frelich, 2011), we instead used biomass at the time of harvesting as a fitness proxy, as biomass was found to be highly correlated with per-capita seed and pollen production in a field study across 39 sites in Europe (Lommen et al., 2017). At the final harvest, we counted the numbers of developing male inflorescences of each plant as a proxy for potential male reproductive output, and we measured soil moisture content (SMC) using a soil moisture sensor (Delta-T Devices) in all pots to assess differences in water use of the plants. We then separated all plants into above-ground and root biomass, dried them at 60°C for 72 hr and weighed them. To further characterize the phenotypic differences in functional traits among the plants, we also calculated NAR, the relative growth rate (RGR), RWC and SLA of each plant (see Appendix S1D for details).

We analysed our data with (generalized) linear mixed models, using the glmer/lmer functions in the R package lme4, which uses maximum likelihood to estimate model parameters (Bates et al., 2014). Days to flowering and number of developing male inflorescences were analysed with glmcr using a log-link and a Poisson distribution, and all other (normally distributed) variables-seed size, germination rate, plant biomass, RWC, SLA, RGR, NAR and SMC—were analysed with lmer. The models included generation and selection treatments (i.e. parent, control offspring and warming offspring) as fixed factors and initial height as a covariate to partially account for maternal effects. As models that include cage as random effect do not differ significantly from models that include cage nested within the field block as random effect ($p \geq .48$), we only used cage as the random effect (Schmid, Baruffol, Wang, & Niklaus, 2017). We adjusted $p$-values using the Bonferroni–Holm method to correct for type 1 error. In cases where significant differences occurred ($p_{adj} < .05$), we carried out Tukey’s post hoc tests using the glht function in the R package multcomp (Hothorn, Bretz, & Westfall, 2008) to compare the treatments of the parents and the two offsprings. We further analysed the relationships between different traits. To check if a relationship was different between cages, we compared random-intercept and random-slope linear mixed-effects models for the trait of interest with cage used as a random effect (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). We compared these two models with log-likelihood ratio tests. The random-slope version was never significantly better than the simpler random intercept model (all $p \geq .53$), which indicates that cages have no significant effect on the tested relationships.

To quantify genetically based phenotypic differentiation among populations ($P_{ST}$) we used the $P_{st}$ function in the R package Pstat (Da Silva & Da Silva, 2018), with 99% confidence intervals from 5,000 bootstrapped estimates. How well $P_{ST}$ approximates $Q_{ST}$ depends on the heritability $h^2$ and the between-population additive genetic component $c$ (Brommer, 2011). Following Sun and Roderick (2019), we set heritability to 0.46–0.95 and $c$ from 0.01 (1% phenotypic variance due to additive genetic effects) to 1 (all phenotypic variance due to additive genetic effects) and calculated $P_{st}$ for this range of $h^2$ and $c$ values. To test a realistic range of possibilities, we thus calculated $P_{st}$ for five values of $c/h^2$ (0.01, 0.5, 1, 1.5, 2).

2.4 | Comparisons of phenotypic and genetic divergence

In microevolutionary studies, phenotypic and genetic analyses provide different, but complementary information. Specifying a change as phenotypic does not imply that the change itself was not genetic, but simply that the relative contribution of genetic and non-genetic effects is not known (Hendry & Kinnison, 1999). Phenotypic and genetic changes can be assessed allochronically when comparing the same population at different points in time (i.e. temporal context; between generations in our study), or synchronically by comparing...
populations that had a common origin in the past (i.e. spatial context or space-for-time approach; between treatments within generations in our study; Merilä & Hendry, 2014; Verheyen, Tüzün, & Stoks, 2019). Allochronic studies are used to estimate the pace of evolutionary change, whereas synchronic studies more appropriately estimate divergence (Hendry & Kinnison, 1999). In the synchronic approach, different populations can be grown together under specific conditions, while an allochronic approach is generally harder to implement, or sometimes impossible (Merilä & Hendry, 2014). Our allochronic approach corresponds to the forward-in-time approach of the resurrection protocol recently described by Franks, Hamann, and Weis (2018). Combining the synchronic and allochronic approach will allow assessing the population differentiation in time and space simultaneously (Van Dijk & Hautekeete, 2014). To infer the adaptive evolution of phenotypic changes in response to climate change, we can think of a synchronic comparison to assess if the rate or directionality of evolutionary change in the selection treatment (allochronic setting) exceeds the one in the control treatment (cf. Figure 1). This approach has been developed and applied in our study.

When attempting to estimate the magnitude of phenotypic differentiation due to selection, one must control for variation among cages caused by genetic drift. For this, we assessed both the molecular and phenotypic changes across generations (i.e. rates of divergence) for each of the five populations of the two treatments. To test whether these data are consistent with a hypothesis of rapid response to selection, we first estimated, in the allochronic comparisons, the rates of phenotypic and sequence divergence, separately for each experimental population and trait. Including the allochronic comparison in our analysis considers the fact that the initial populations were similar (same maternal families), but not identical due to high to complete outcrossing of ragweed (Friedman & Barrett, 2008; Li et al., 2012). To estimate rates of phenotypic divergence (Figure 1) we used the Haldane metric (Gingerich, 1983):

$$P_{ij} = \frac{|p_i - p_j|}{\sigma_p^2 T},$$

where t represents a specific trait and j is either control offspring or warming offspring, and $p_i$ is the trait mean for parents, $\sigma_p^2$ is the variance among the parental generation and T is the number of generations between the two populations compared. Similarly, rates of sequence divergence ($G_{ij}$ for control and warming; respectively, Figure 1) were estimated as:

$$G_{ij} = \frac{1}{n} \sum f_i - f_j \sigma_i^2 T,$$

where n is the length of the DNA sequence examined, $f_i$ is the allele frequency of SNP i for parents, j is either control offspring or warming offspring and $f_j$ is the allele frequency of SNP i for either control offspring or warming offspring, $\sigma_i^2$ is the variance of the allele frequencies of the SNP i in the parents and T is the number of generations between the two populations compared. In a second step, we then calculated the ratios between the rates of phenotypic and sequence divergence as:

$$PG_{ij} = \log \left( \frac{P_{ij}}{G_{ij}} \right).$$

A larger value of the ratio will indicate stronger selection on phenotypes (Merilä & Hendry, 2014), and the comparison between the ratio with and without warming selection can thus indicate the relative strength of natural selection in the experimental warming treatments. We expected the $PG_{w}$ for warming treatment to be larger than the $PG_{c}$ for control if the simulated warming caused indeed stronger genetic response to selection than under control. For each of the nine traits, we compared the PG ratio between treatments with ANOVA. p-values were then adjusted to correct for type 1 errors.

We assessed normality of the residuals of all models using QQ-plots and found that all residuals were normally distributed. All statistical analyses were done in R version 3.4.3 (R Development Core Team, 2017), and all figures created with the R package ggplot2.

**FIGURE 2** Genomic differentiations between generations and treatments. (a) Principal component analysis visualizing genetic differences, using all polymorphic SNP markers, among the 10 experimental populations of Ambrosia artemisiifolia, with grey, blue and red symbols indicating parental, control offspring and warming offspring respectively. The ellipses represent 95% confidence interval. (b) Cross-generation population differentiation ($F_{ST}$) for the five blocks of the two treatments, with blue $F_{ST}$ distributions representing differentiation between control offspring and their parents, and red distributions for differentiation between warming offspring and their parents. Vertical dotted lines indicate the peak and asterisks the medians of the distributions.
3 | RESULTS

Based on pool-seq data, the PCA visualizing the genetic similarities of all parent and offspring populations showed a much stronger separation between the two generations under warming conditions than under control conditions, and greater similarity among populations within both control offspring and warming offspring as compared with their respective parental populations (Figure 2a). In both the control and warming treatment offspring populations had a significantly lower $\pi$ (genetic variability within population) than their parental populations ($p < .001$). The cross-generation $F_{ST}$ values were significantly higher for warming populations than for control populations ($p < .001$; Figure 2b), indicating stronger genetic divergence from the parental generation under warming conditions.

![Phenotypic differentiations between generations and treatments.](image)

**FIGURE 3** Phenotypic differentiations between generations and treatments. Phenotypic differences between parental and offspring plants of *Ambrosia artemisiifolia* from the two experimental treatments, when compared in a common environment. The error bars are ±1SE. The letters on top indicate significant differences at $p_{adj} < .05$.

![Phenotypic differentiation among populations and comparisons of phenotypic versus genetic divergence.](image)

**FIGURE 4** Phenotypic differentiation among populations and comparisons of phenotypic versus genetic divergence. (a) Phenotypic differentiation ($P_{ST}$) among experimental *Ambrosia artemisiifolia* populations from the control versus warming treatment. The values are $P_{ST}$ estimates for $c/h^2 = 1$, with 99% bootstrap confidence intervals (see Figure S4 for $P_{ST}$ estimates under different $c/h^2$ assumptions). (b) Ratios of phenotypic to genetic divergence rates across two generations for *A. artemisiifolia* populations subjected to warming versus control conditions, with blue and red points for control and warming treatment populations; the transparent open squares give the mean with error bars (±1SE). Asterisks indicate significant differences at $p_{adj} < .01$ (***) and $p_{adj} < .05$ (**).
We found significant variation among maternal families for all nine phenotypic traits in the parental generation (Figure S2; all $F ≥ 1.71, p_{adj} ≤ .03$), indicating substantial genetic variation for selection to act upon. When we tested for phenotypic changes between generations and treatments, we found significant changes in days to flowering ($\chi^2 = 14.39, p_{adj} = .001$), the total biomass of ragweed ($\chi^2 = 9.23, p_{adj} = .01$) and a marginally significant change in the number of developing male inflorescences ($\chi^2 = 5.29, p_{adj} = .06$; Table S1; Figure 3). Offspring plants from the warming treatment flowered significantly later and produced larger total biomass than control offspring, or than parental plants ($\text{glht} \text{ tukey } p ≤ .04$), but there were no differences between offspring control and parental plants ($\text{glht} \text{ tukey } p = .76$; Figure 3b). For seed traits, that is, seed size and seed germination rate ($\chi^2 ≤ 1.26, p_{adj} ≥ .26$) and all other plant phenotypic traits ($\chi^2 ≤ 2.40, p_{adj} ≥ .27$; Table S1), there were no significant differences between generations and treatments. There was a significant negative correlation between total plant biomass and SMC for parental plants and control offspring (both $p_{adj} < .001$, $r^2 ≥ .18$), but not for warming offspring ($p_{adj} = .33, r^2 = .02$; Figure S3).

In seven out of nine phenotypic traits, offspring from ragweed populations that experienced warming had significantly lower $P_{ST}$ value, that is, a lower phenotypic differentiation among replicate populations, than offspring from control populations (Figure 4a; Figure S4 for testing different $c/h^2$ assumptions). In two of the nine studied phenotypic traits—days to flowering and total biomass—we found significant differences between control and warming populations in their ratio of phenotypic to genetic differentiation, and in both cases the ratio was substantially higher for warming populations ($PGW > PGC; \chi^2 ≥ 7.82, p_{adj} ≤ .005$; Figure 4b).

4 | DISCUSSION

Forecasting the ecological and evolutionary consequences of the interaction between plant invasion and climate change requires a thorough understanding of the underlying biological processes in plant invaders. We know from previous studies that environmental change can result in rapid selection and evolution of plant phenotypes. For instance, Skroppa and Kohmann (1997) demonstrated adaptation of Norway spruce (Picea abies) to local climatic conditions after only one generation, and Franks, Sim, and Weis (2007) found rapid evolution of flowering time in the annual Brassica rapa in response to climate change after just three generations. More recently, Nguyen et al. (2016) showed earlier flowering in two invasive plant species in response to selection over fewer generations of water manipulations, and Nowak et al. (2018) demonstrated adaptive evolution of the Alpine Pennycress Noccaea caerulescens after one generation of exposure to various levels of zinc contamination in the soil. Due to multiple introductions and admixtures between differentiated populations, either prior or post-introduction, invasive plants can exhibit large within-population genetic diversity (Lavergne & Molofsky, 2007). This has also been found for ragweed in Europe (van Boheemen et al., 2017; Gallien et al., 2016; Genton et al., 2005). Hahn and Rieseberg (2017) report considerable heterosis effects among particular native common ragweed population crosses, especially under simulated herbivory, but not so in crosses from the introduced French population, possibly indicating that these populations were already admixed and benefit little from further mixing. This has recently been confirmed by van Boheemen et al. (2017), who showed that a historical admixture zone within native North America originated before global invasion of this weed and that European ragweed populations most likely established through multiple introductions from the native range, including those from admixed populations. This resulted in rapid adaptation of life-history traits including size and flowering time to climate (latitude) in Europe (van Boheemen et al., 2019; McGoe, Hodgins, & Stinchcombe, 2020) and thus illustrates the potential for rapid adaptation to environmental changes in ragweed. McGoe and Stinchcombe (2018) recently reported significant quantitative genetic variation for a range of traits in European ragweed populations. To bolster the within population genetic variation in our experimental study, we used maternal plants from multiple populations across Northern Italy. We thus expect that the evolutionary changes observed in our study also reflect the adaptive potential of populations in the introduced range.

One component for such evolutionary changes in invasive species can be the increased genetic variation and of novel or transgressive phenotypes created through admixtures (Bock et al., 2015; van Boheemen et al., 2019; Rius & Darling, 2014). In addition, a small number of loci with large phenotypic effects (Lendvai & Levin, 2003), or strong directional selection (Rego, Messina, & Gompert, 2019) as in our study, may further contribute to rapid evolution. Here we found significant genetic divergence in several phenotypic traits of ragweed populations under warming selection. Since both seeds collected from the parents and the second-year seedlings are purely first generation, our results demonstrate evolutionary changes within a single generation.

The starting material for our selection experiment were 60 seed families from 19 invasive ragweed populations collected across Northern Italy. We found significant phenotypic variation among seed families for all studied traits, which corroborates previous reports of substantial genetic variation within introduced European populations (van Boheemen et al., 2017; Genton et al., 2005). Most importantly, it confirms that our experimental populations initially harboured plenty of raw material for selection to act upon, and that our artificial populations well reflect the high genetic within population variation described for this area (van Boheemen et al., 2017).

Pool-sequencing the experimental populations over two generations showed that in contrast to control populations, warming populations had higher $F_{ST}$ values and greater genetic differentiation between parental and offspring generations. These results show that our experimental warming treatment strongly accelerated evolutionary change. In addition, the populations for both the control and warming treatment became more similar within the offspring generation (smaller ellipses) as compared with the parental generation, indicating directional selection in both treatments.
The experimental populations not only evolved at the level of DNA sequence, but also at the level of phenotype. Under warming conditions, plants became larger and they flowered later, which might indicate selection towards faster growth and larger biomass accumulation, a syndrome of more efficient resource use, resulting in increased reproductive output (Lommen et al., 2017). van Boheemen et al. (2019) recently examined divergence of life-history traits in relation to climate in native North American versus introduced European and Australian populations of ragweed, and found that climate change is likely to select for larger ragweed plants that flowered later, which is in line with our results. Similarly, McGoe et al. (2020) found that larger and later-flowering ragweed plants are associated with warmer climates and lower latitudes in both North America and Europe. It is very likely that the phenotypic changes observed in our warming treatment are also the result of natural selection. First, all five replicates of each treatment showed consistent genetic changes in similar directions (Figure 2a). Second, we found no genetic separation between generations for the control populations, and thus increased genetic drift alone cannot explain the results. Third, we found significantly higher phenotypic to genomic differentiation ratios (PG ratio) in the warming treatment than in the control treatment exactly for the two divergent traits, flowering time and total biomass. Genetic response to selection can generally occur through selection on standing variation, but also through mutation and recombination. As we found that rapid genetic responses to selection. We show that invasive ragweed populations are in line with our above DNA sequencing results and the reduced $P_{ST}$ for seven out of nine traits among the warming populations. Our results therefore demonstrate that climate change, as simulated by our warming treatment, can have rapid effects on the genetic composition of ragweed populations. Selection for particular genotypes may allow some traits to become more prominent in the mid- to longer term. Such evolutionary changes were recently found to be responsible, at least partially, for niche shifts in the introduced European range of ragweed (van Boheemen et al., 2019; Gallien et al., 2016). Moreover, larger ragweed plants, as selected for in our warming treatment, have previously been found to have higher per-capita seed and pollen production (Lommen et al., 2017). Larger plants are thus expected to further increase the future spread and impact of ragweed under changing climatic conditions.

**5 | CONCLUSIONS**

Adaptive evolution over short timescales is well-documented in invasive species (Prentis et al., 2008), specifically also for common ragweed (van Boheemen et al., 2019; Chun et al., 2011; Gallien et al., 2016; McGoe et al., 2020). Our approach combining comparisons between generations (allochronic) and between treatments (synchronic) in an experimental evolutionary field study provided a powerful test for rapid genetic responses to selection. We show that invasive ragweed populations may rapidly evolve towards larger biomass accumulation under conditions of climate warming, and that such changes can take place within a single generation. Our results also indicate that this was mainly a consequence of differential mortality, growth or reproduction among the initially sown genotypes. Short-term evolutionary responses to climate change may aggravate the impact of some plant invaders in the future and should be considered when making predictions about future distributions and impact of plant invaders.

**ACKNOWLEDGEMENTS**

Y.S. was supported by an Advanced Postdoc.Mobility fellowship from the Swiss National Science Foundation (SNSF; Project No. P300PA_
DATA AVAILABILITY STATEMENT
The data that support the findings of this study are openly available in Zenodo: https://zenodo.org/record/3947913, 3948037, 3948090, 3948094, 3948098, 3948140, 3948142, 3948326, 3948328.

ORCID
Yan Sun https://orcid.org/0000-0002-6439-266X

REFERENCES
Agrawal, A. A., Hastings, A. P., Johnson, M. T. J., Maron, J. L., & Salminen, J. P. (2012). Insect herbivores drive real-time ecological and evolutionary change in plant populations. Science, 338(6103), 113-116. https://doi.org/10.1126/science.1225977
Alberto, F. J., Atiken, S. N., Alia, R., González-Martínez, S. C., Hänninen, H., Kremer, A., ... Savolainen, O. (2013). Potential for evolutionary responses to climate change—Evidence from tree populations. Global Change Biology, 19(6), 1645-1661. https://doi.org/10.1111/gcb.12181
Alexander, J. M., & Edwards, P. J. (2010). Limits to the niche and range margins of alien species. Oikos, 119(9), 1377-1386. https://doi.org/10.1111/j.1600-0706.2009.17977.x
Barghi, N., Tobler, R., Nolte, V., Jakšić, A. M., Mallard, F., Otte, K. A., ... Schlötterer, C. (2019). Genetic redundancy fuels polygenic adaptation in Drosophila. PLoS Biology, 17(2), e3000128. https://doi.org/10.1371/journal.pbio.3000128
Barrs, H., & Weatherley, P. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. Australian Journal of Biological Sciences, 15(3), 413-428. https://doi.org/10.1071/BI9620413
Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., ... Grothendieck, G. (2014). Package ‘lme4’. R foundation for statistical computing. Vienna, 12.
Lavergne, S., & Molofsky, J. (2007). Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences of the United States of America*, 104(10), 3883–3888. https://doi.org/10.1073/pnas.0607324104

Lavergne, S., Mouquet, N., Thuiller, W., & Ronce, O. (2010). Biodiversity and climate change: Integrating evolutionary and ecological responses of species and communities. *Annual Review of Ecology, Evolution, and Systematics*, 41, 321–350. https://doi.org/10.1146/annurev-ecolsys-102209-144628

Lendvai, G., & Levin, D. (2003). Rapid response to artificial selection on flower size in *Phlox. Hereditas*, 90(4), 336–342. https://doi.org/10.1083/js.jhdy.6800249

Li, J., Du, L., Guan, W., Yu, F., & van Kleunen, M. (2016). Latitudinal and longitudinal clines of phenotypic plasticity in the invasive herb *Solidago canadensis* in China. *Oecologia*, 182(3), 755–764. https://doi.org/10.1007/s00442-016-3699-x

Li, X., Liao, W., Wolfe, L. M., & Zhang, D. (2012). No evolutionary shift in the mating system of North American *Ambrosia artemisiifolia* (Asteraceae) following its introduction to China. *PLoS One*, 7(2), e31935. https://doi.org/10.1371/journal.pone.0031935

Lommen, S. T. E., Hallman, C. A., Jongejans, E., Chauvel, B., Leitsch-Vitalos, M., Aleksanyan, A., ... Müller-Schärer, H. (2017). Explaining variability in the production of seed and allergenic pollen by invasive *Ambrosia artemisiifolia* across Europe. *Biological Invasions*, 20(6), 1475–1491. https://doi.org/10.1007/s10530-017-1640-9

Lommen, S. T., Jongejans, E., Leitsch-Vitalos, M., Tokarska-Guzik, B., Zalai, M., Müller-Schärer, H., & Karrer, G. (2018). Time to cut: Population models reveal how to manage invasive common ragweed cost-effectively. *NeoBiota*, 39, 53–78. https://doi.org/10.3897/neobiota.39.23398

Maisto, G., Santoro, L., & Arena, C. (2013). Heavy metal accumulation in leaves affects physiological performance and litter quality of *Quercus ilex* L. *Journal of Plant Nutrition and Soil Science*, 176(5), 776–784. https://doi.org/10.1002/jpln.201200053

Marion, G. M., Henry, G., Freeckman, D. W., Johnstone, J., Jones, G., Jones, M. H., ... Virginia, R. A. (1997). Open-top designs for manipulating field temperature in high-latitude ecosystems. *Global Change Biology*, 3(51), 20–32. https://doi.org/10.1111/j.1365-2486.1997.tb0136x

McGoey, B. V., Hodgins, K. A., & Stinchcombe, J. R. (2020). Parallel flowering time clines in native and introduced ragweed populations are likely due to adaptation. *Ecology and Evolution*, 10, 4595–4608. https://doi.org/10.1002/ece3.6163

McGoey, B. V., & Stinchcombe, J. R. (2018). Introduced populations of ragweed show as much evolutionary potential as native populations. *bioRxiv*, 305540. https://doi.org/10.1101/305540

Merlii, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications*, 7(1), 1–14. https://doi.org/10.1111/eva.12137

Merow, C., Bois, S. T., Allen, J. M., Xie, Y., & Silander, J. A. (2017). Climate change both facilitates and inhibits invasive plant ranges in New England. *Proceedings of the National Academy of Sciences of the United States of America*, 114(16), E3276–E3284. https://doi.org/10.1073/pnas.1609633114

Moloney, K. A., Holzapfel, C., Tielbörger, K., Jeltsch, F., & Schurr, F. M. (2009). Rethinking the common garden in invasion research. *Perspectives in Plant Ecology, Evolution and Systematics*, 11(4), 311–320. https://doi.org/10.1016/j.ppees.2009.05.002

Moran, E. V., & Alexander, J. M. (2014). Evolutionary responses to global change: Lessons from invasive species. *Ecology Letters*, 17(5), 637–649. https://doi.org/10.1111/ele.12262

Mousseau, M. (1999). At the crossroads of plant physiology and ecology. *Trends in Ecology & Evolution*, 14, 163–167. https://doi.org/10.1016/S1365-2486.2010.02294.x

Mullan, D., & Pietragalla, J. (2012). Leaf relative water content. In A. Pask, J. Pietragalla, D. Mullan, & M. P. Reynolds (Eds.), *Physiological breeding II: A field guide to wheat phenotyping* (pp. 25–27). Mexico, D.F.: CIMMYT.

Müller-Schärer, H., Bouchenouss, S., Litto, M., McEvoy, P. B., Roderick, G. K., & Sun, Y. (2020). How to better predict long-term benefits and
risks in weed biocntrol: An evolutionary perspective. Current Opinion in Insect Science, 38, 84–91. https://doi.org/10.1016/j.cois.2020.02.006

Müller-Schärer, H., Sun, Y., Chauvel, B., Karrer, G., Kazinczi, G., Kudsk, P., ... Lommen, S. (2018). Cross-fertilizing weed science and plant invasion science to improve efficient management: A European challenge. Basic and Applied Ecology, 33, 1–13. https://doi.org/10.1016/j.baae.2018.08.003

Nadeau, C. P., & Urban, M. C. (2019). Eco-evolution on the edge during climate change. Ecosystems, 42(7), 1280–1297. https://doi.org/10.10111/ecog.04404

Nguyen, M. A., Ortega, A. E., Nguyen, K. Q., Kimball, S., Goulden, M. L., & Roach, D. A., & Wulff, R. D. (1987). Maternal effects in plants. Heredity, 17(5), 1074–1084. https://doi.org/10.1038/hdy.1987.86

van Boheemen, L. A., Atwater, D. Z., & Hodgins, K. A. (2019). Rapid and repeated local adaptation to climate in an invasive plant. New Phytologist, 222(1), 614–627. https://doi.org/10.1111/nph.15564

Vilà, M., Espinar, J. L., Hejda, M., Hulme, P. E., Jarošík, V., Maron, J. L., ... Pyšek, P. (2011). Ecological impacts of invasive alien plants: A meta-analysis of their effects on species, communities and ecosystems. Ecology Letters, 14(7), 702–708. https://doi.org/10.1111/j.1461-0248.2011.01628.x

Zuur, A., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). Mixed effects models and extensions in ecology with R. New York, NY: Springer Science & Business Media. https://doi.org/10.1007/978-0-387-87458-6

SUN ET AL.

How to cite this article: Sun Y, Bosdorf O, Grados RD, Liao Z, Müller-Schärer H. Rapid genomic and phenotypic change in response to climate warming in a widespread plant invader. Glob Change Biol. 2020;00:1–12. https://doi.org/10.1111/gcb.15291

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.