RAPID AND REPEATED LOCAL ADAPTATION TO CLIMATE IN AN INVASIVE PLANT

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SUMMARY

- Biological invasions provide opportunities to study evolutionary processes occurring over contemporary timescales. To explore the speed and repeatability of adaptation, we examined the divergence of life-history traits to climate, using latitude as a proxy, in the native North American and introduced European and Australian ranges of the annual plant *Ambrosia artemisiifolia*.
- We explored niche changes following introductions using climate niche dynamic models. In a common garden, we examined trait divergence by growing seeds collected across three ranges with highly distinct demographic histories. Heterozygosity-fitness associations were used to explore the effect of invasion history on potential success. We accounted for non-adaptive population differentiation using 11,598 SNPs.
- We revealed a centroid shift to warmer, wetter climates in the introduced ranges. We identified repeated latitudinal divergence in life-history traits, with European and Australian populations positioned at either end of the native clines.
- Our data indicate rapid and repeated adaptation to local climates despite the recent introductions and a bottleneck limiting genetic variation in Australia. Centroid shifts in the introduced ranges suggest adaptation to more productive environments, potentially contributing to trait divergence between the ranges.

KEYWORDS

Invasion, trait evolution, climate niche dynamics, local adaptation, latitudinal clines, climate adaptation, heterozygosity fitness correlations

INTRODUCTION

During biological invasions species commonly spread over large and climatically diverse geographic areas. In doing so, they often re-establish within climatic niches found in their native ranges, and in some cases flourish in new environments (Sax & Brown, 2000; Allendorf & Lundquist, 2003; Atwater *et al.*, 2018). Although plasticity and broad ecological tolerance may facilitate the spread of invaders across such heterogeneous climates (e.g. Geng *et al.*, 2007; Zhang *et al.*, 2010), a growing number of empirical examples suggest that rapid adaptation to local conditions can also enable the establishment and spread of these species in the face of new selective dynamics (Huey *et al.*, 2000; Lachmuth *et al.*, 2011; Colautti & Barrett, 2013; Chown *et al.*, 2014; Turner *et al.*, 2014; Oduor *et al.*, 2016). As such, invasions provide an opportunity to study contemporary adaptive processes, which is key in an era of rapid, human-induced, environmental change. As many single species have invaded several distinct regions of the globe, comparisons of the native range to multiple successful introductions could illuminate if and when traits evolve in parallel along climatic gradients (Moran & Alexander, 2014).

Climate is known to be an important selective factor shaping a diverse array of plant traits from physiological (e.g. Maron *et al.*, 2007; Ordoñez *et al.*, 2009), to life history traits (e.g. Franks *et al.*, 2007; Nakazato *et al.*, 2008; Colautti & Barrett, 2013) to defence (e.g. Moles *et al.*, 2011; Colomer□Ventura *et al.*, 2015). For instance, specific leaf area commonly increases with latitude (Frenne *et al.*, 2013), likely reflecting adaptation to latitudinal changes in temperature, precipitation, light availability, or herbivory (Poorter *et al.*, 2009). Furthermore, trade-offs among life history traits can shape adaptive trait divergence in response to local conditions and impact the evolutionary trajectory of trait combinations in invasive populations (Etterson & Shaw, 2001; Griffith & Watson, 2005; Colautti *et al.*, 2010; Hodgins & Rieseberg, 2011; Colautti & Barrett, 2013). Reductions in season-length at higher latitudes are frequently reported to select for early flowering at the cost of diminished plant size (Colautti *et al.*, 2010; Li *et al.*, 2014). Coordinated shifts in life-history traits along latitudinal gradients within ranges have been documented in several invasive plants (Dlugosch & Parker, 2008b; Hodgins & Rieseberg, 2011; Colautti & Barrett, 2013).

Latitudinal patterns in plant size could have important evolutionary consequences for other plant traits. Variation in plant size can influence optimal

resource allocation to male and female sex function (Charnov, 1982; De Jong & Klinkhamer, 1989; Klinkhamer *et al.*, 1997). In wind-pollinated plants, height can affect fitness returns directly through more effective pollen dispersal in taller plants (Burd & Allen, 1988; Klinkhamer *et al.*, 1997), as well as indirectly through increased availability of resources (Lloyd & Bawa, 1984; De Jong & Klinkhamer, 1989; Klinkhamer *et al.*, 1997; Zhang, 2006). Outcrossing wind-pollinated plants are predicted to adaptively change sex allocation to be more male-biased with increase in size (Lloyd, 1984; De Jong & Klinkhamer, 1989; de Jong & Klinkhamer, 1994; Klinkhamer *et al.*, 1997), as local seed dispersal should lead to saturating female gain curves (Lloyd & Bawa, 1984; Sakai & Sakai, 2003), yet more linear male function gain curves are expected (Klinkhamer *et al.*, 1997; Friedman & Barrett, 2009). Latitudinal clines in height could therefore be expected to lead to adaptive shifts in sex allocation. However, studies investigating the evolution of sex allocation patterns over wide geographic ranges are rare (Guo *et al.*, 2010; Barrett & Hough, 2012).

Many factors could impact trait evolution of native and invasive populations evolving in response to similar climatic gradients resulting in divergent outcomes. For instance, demographic events, such as bottlenecks, genetic drift in founding populations and admixture could differentially affect native and invasives' adaptive capacity or influence the route by which adaptive evolution proceeds (Lee, 2002; Facon *et al.*, 2006; Prentis *et al.*, 2008; Rius & Darling, 2014; Bock *et al.*, 2015; Estoup *et al.*, 2016; Hodgins *et al.*, 2018). Although the impacts of bottlenecks during colonization on molecular variation are well characterized its effect on quantitative trait variation are not well established (Dlugosch & Parker, 2008a; Dlugosch *et al.*, 2015a). Moreover, even if the required genetic variation is present in the introduced range, the probability of observing trait clines in the introduced range depends on the time since introduction and the strength of climate-mediated selection (Prentis *et al.*, 2008; Bock *et al.*, 2015). Shifts in the biotic environment associated with introduction may also influence the evolution of plant traits, potentially impacting trait clines. Indeed, the impact of the biotic environment has been commonly considered in the examination of trait evolution in invasive plant species (Felker□Quinn *et al.*, 2013). By contrast, the invasion history could create patterns of trait variation (e.g., through climate matching (Maron *et al.*, 2004)) mimicking adaptive population divergence (Colautti *et al.*, 2009; Colautti & Lau, 2015). Therefore careful consideration of the source populations is required during studies examining adaptive trait evolution

during invasion. Dissection of these various mechanisms is required to advance our understanding of the role of rapid evolution in invasive spread (Keller *et al.*, 2009; Bonhomme *et al.*, 2010; Lachmuth *et al.*, 2011; Agrawal *et al.*, 2015; Cristescu, 2015; Dlugosch *et al.*, 2015a).

We examine the repeatability and divergence of important life-history traits in the native North American and introduced European and Australian ranges of *Ambrosia artemisiifolia*. We raised seeds collected at 77 locations from broad climatic scales in a common garden and accounted for non-adaptive genetic differentiation using 11,598 genotype-by-sequencing SNPs, as neutral processes could impact trait variation. We here focus on evolution of plant size (height, biomass and growth), reproductive traits (flowering onset, dichogamy, sex function allocation, seed weight, total and relative reproductive biomass) and physiology (specific leaf area). We investigate four specific questions: *1) Do native and introduced populations occur in similar climates niches?* As climate is likely important in governing trait variation in this species, we examined climatic niche shifts following introduction to assess how traits might be predicted to diverge within and among the ranges. *2) Is there evidence for rapid parallel adaptation to latitude?* Repeatable trait clines for each range along latitudinal gradients, highly associated with many aspects of climate, would provide strong evidence that rapid adaptation to similar selective environments has occurred. We additionally explore coordinated shifts in traits potentially linked by trade-offs. *3) Is there evidence for trait differentiation between native and introduced ranges?* By examining patterns across multiple introduced ranges, we can explore if novel recipient communities generated trait divergence during introduction, or if adaptation to local climates dominates patterns of trait variation. *4) Is there a correlation between heterozygosity and fitness related traits?* Significant correlations would reveal if demographic changes such as bottlenecks, admixture and inbreeding have likely impacted the evolution of traits during this species' extensive range expansion. Such correlations are predicted at the individual and population level in regions that have recently expanded their range, including those that have undergone admixture (Peischl & Excoffier, 2015).

MATERIAL AND METHODS

Study species

Ambrosia artemisiifolia is a wind pollinated, outcrossing, hermaphrodictic

annual, which has aggressively spread from its native North America to many regions worldwide (Laaidi *et al.*, 2003; Smith *et al.*, 2013). The first records documenting the invasion are in central France around 1850 (Chauvel *et al.*, 2006). Multiple introductions from distinct native sources ensued to both east and west Europe, resulting in levels of genetic variation equivalent to those found in the native range (Chun *et al.*, 2010; Gladieux *et al.*, 2010; Gaudeul *et al.*, 2011; van Boheemen *et al.*, 2017). Genetic analysis suggests the Australian populations originate from a subsequent single introduction event around 80 years ago, derived from the European introduction, although the exact source is unknown (van Boheemen *et al.*, 2017). Range expansion likely occurred both north- and southward following this south-Queensland introduction (Palmer & McFadyen, 2012; van Boheemen *et al.*, 2017)

Latitudinal clines in phenology have been observed within the native North American range and the introduced ranges of Europe (Chun *et al.*, 2011; Leiblein-Wild & Tackenberg, 2014) and China (Li *et al.*, 2014), with earlier reproduction and greater relative investment in reproductive biomass in high-latitudinal compared to low-latitudinal populations (Chun *et al.*, 2011; Hodgins & Rieseberg, 2011; Li *et al.*, 2014). The wind-spread pollen is a major cause of hayfever worldwide and its medical treatment costs millions of dollars each year (Taramarcaz *et al.*, 2005), providing considerable incentive to understand the factors impacting pollen production in this species.

Climate niche dynamics

To estimate the climatic niche occupied by *A. artemisiifolia* in its native North American, introduced Eurasian and Australasian ranges, we used ordination-based species distribution models. Models were taken from a larger study of 835 species (Atwater *et al.*, 2018), where the methods are described in detail. Briefly, occurrence data were collected from the Global Biodiversity Information Facility and plotted in 2-dimensional climate space based on rotated component variables (RCA1: temperature and RCA2: precipitation) of the 19 WorldClim variables (Hijmans *et al.*, 2005) representing annual trends, seasonality and means in temperature or precipitation (Table S1, Supplementary Material). We selected these variables as they are commonly used in studies on species distribution and local adaptation including for studies of *A. artemisiifolia* (e.g. Leiblein-Wild & Tackenberg, 2014; Sun *et al.*, 2017). We next generated a climatic occupancy map that described the occurrence

probability of *A. artemisiifolia* as it varied among climates. Next, we divided this occupancy map by a map of sampling bias, as estimated from a dataset of 815 terrestrial plant species (Atwater *et al.*, 2018). Removal of both geographic and climatic sampling bias in this way produced bias-corrected estimates of occurrence probability in each set of climatic variables, for each of the three ranges (North American, Eurasian, and Australasian). Finally, these predictions were smoothed using We used Monte Carlo resampling (n = 120) to compare observed niche dynamics to those expected using randomly resampled occurrence points (Atwater *et al.*, 2018). We used Schoener's *D* (Schoener, 1968) to estimate niche overlap between the native range and each introduced range (*i.e.* the degree of similarity in occupancy probabilities between both ranges), and we estimated niche stability (the proportion of climates occupied by the species in both their introduced an native range), expansion (the proportion of climates occupied in the introduced range that are available but unoccupied in the native range), and unfilling (the proportion of climates occupied in the native range that are available but unoccupied in the introduced range)(Guisan *et al.*, 2014). Finally, we tested whether the location of the niche centroid differed between native and introduced ranges.

Data collection

To investigate local environments, we described climatic differences between 27 populations in the native range of North America, 32 populations in the introduced European range, and 18 populations in the introduced Australian range (Fig. S1, Supplementary Material). We used the 19 WorldClim variables and added a geographic dimension to the data by including altitude, latitude and longitude, as these variables are shown to be important in *A. artemisiifolia* growth and fitness (Chun *et al.*, 2011; Chapman *et al.*, 2014). To explore associations between climatic and geographic variables in the sampled populations, we applied a principal component analysis (PCA). We opted to present latitudinal trait clines only, as *i)* the primary principle component was highly correlated with latitude (Fig. S2); *ii)* precipitation and temperature variables were strongly associated with latitude (Spearman's ρ^2=0.326-0.417 (Table S2); *iii)* exploratory analyses revealed associations between each trait and many climatic and geographic variable were highly similar to trait~latitude trends (results not shown); and *iv*) is shown to affect *A. artemisiifolia* season length and photoperiod (Ziska *et al.*, 2011).

To assess the potential for adaptive differentiation along latitudinal gradients, we measured trait variation in a common garden of raised seeds collected at broad geographical scales across the three ranges. This is a traditional approach to detect genetic differences among populations (e.g. Bossdorf *et al.*, 2005; Colautti *et al.*, 2009; Hodgins & Rieseberg, 2011; Savolainen *et al.*, 2013; de Villemereuil *et al.*, 2016). We collected seeds in 2013-2014 and randomly selected an average of 12 maternal families with 20 seeds per family from each population (for a full description of data collection methods, see Supplementary Methods). Following a 6-week stratification at 4°C (Willemsen, 1975), we placed seeds in a 30°C germination chamber with 12h light/dark cycle. Two weeks after germination, we planted a randomly selected seedling from each maternal line in a random order into 100ml kwikpot trays with Debco Seed Raising Superior Germinating Mix. We top-watered all plants and artificially manipulated daylight following the light cycle at 47.3°N (median latitude over all sampling locations). One month later, we performed a second transplant (hereafter day 0) to 0.7L pots with Debco mix and 1.5ml slow-release fertilizer (Osmocote Pro, eight to nine months). We examined variation in life-history traits including growth, phenology and vegetative and reproductive allocation (Table 1).

To assess neutral genetic differentiation underlying trait variation resulting from non-adaptive evolutionary processes, we extracted DNA from leaf tissue of 861 individuals and performed double-digest genotype-by-sequencing library preparation (see Supplementary Methods). We aligned and filtered raw sequences following van Boheemen *et al.* (2017). Briefly, SNPs were aligned using BWA-mem (Li & Durbin, 2009) to a draft reference genome for *A. artemisiifolia* (van Boheemen *et al.*, 2017). We called variants with GATK HaplotypeCaller and filtered SNPs using GATK hard-filtering recommendations (McKenna *et al.*, 2010; Van der Auwera *et al.*, 2013). We identified a total of 11,598 polymorphic biallelic SNPs with 50% SNP call rate. We inferred population genetic structure and calculated individual and population level q-scores for the most likely number of clusters K (=2) with the Bayesian clustering method STRUCTURE v2.3.4 (Pritchard *et al.*, 2000). We used these STRUCTURE q-values as a measure of genetic and, therefore, trait differentiation resulting from non-adaptive (neutral) mechanisms.

Statistical analyses

We conducted all statistical analyses in R v3.4.3 (R Core Team, 2017). We improved normality and reduced heteroscedacity of the data by square root or log-transforming traits where appropriate. We tested all univariate linear mixed models using the lme4 package (Bates *et al.*, 2014). All models tested responses of the following traits: maximum plant height, total biomass, maximum growth rate, flowering onset, dichogamy, floral sex allocation, weight per seed, total and relative reproductive biomass and specific leaf area (Table 1).

To explore patterns of latitudinal trait divergence among ranges, potentially indicative of local adaptation, we tested population mean trait responses to range (native North America, introduced Europe and Australia), latitude, their interaction and latitude^2 (to account for non-linear trait responses) in multi- and univariate models ((M)ANCOVA). In these analyses, latitude values are not randomly distributed among ranges due to the geographic distribution of *A. artemisiifolia*, suggesting a violation of independence (Miller & Chapman, 2001). However, the values of the covariate (latitude) are observational and not manipulated by the independent variables (range) and the (M)ANCOVA assumptions are thus not violated (Keppel, 1991). We increased the power of the multivariate analysis (Scheiner, 2001) by removing highly correlated traits (Spearman's $\rho^2>0.6$, Table S3, Supplementary Material) and calculated the approximate F-statistics and Wilks' λ (multivariate F-value) to measure the strength of the associations. To account for demographic history in patterns of trait divergence in the univariate mixed models, we included population mean STRUCTURE q-scores as a random effect. Here, we calculated significance of fixed effects using type III Wald F tests with Kenward-Roger's approximation of denominator degrees of freedom and step-wise removed non-significant ($p>0.05$) fixed effects using the lmerTest package (Kuznetsova *et al.*, 2015), starting with the highest order interactions. To reduce false discovery of associations due to the number of traits being tested, we 'fdr' corrected p-values (hereafter q-values) of the combined final models using the p.adjust function, further reducing models when applicable.

To explore differences in latitudinal trait clines between ranges as revealed in the ANCOVAs, we tested for significant two-way interactions between range and latitude which is indicative of non-parallel trait~latitude slopes among the ranges. To further dissect the extent of trait divergence and its dependence on latitude, we

compared ANCOVA model estimates of traits in the introduced ranges to the native estimates at the minimum and maximum observed latitude in each range, where applicable (EU_{min}, NA_{max}, NA_{min} and AU_{max}) (Fig. S4, Supplementary Material). We tested overall pairwise range differences in trait values for significant range effects, when higher order interactions involving range were not significant. We explored the highest order significant interactions, using χ^2 tests with Holm p-value correction using the phia package (De Rosario-Martinez, 2013).

We verified the presence of the well-described plant height-flowering time trade-off and examined associated patterns in other reproductive traits (dichogamy and sex allocation) within ranges. We tested linear relations between individual trait values of control treated plants in mixed models, with plant height, range and their interaction as fixed effects. In addition to individual STRUCTURE q-values, we added population as random effect. As above, we calculated fixed effects significance with type III Wald F tests, step-wise removing non-significant fixed effects. A significant interaction between range and height indicated a differential slope between the focal reproductive trait and plant height between ranges. We explored the highest order significant interactions using χ^2 tests with Holm p-value correction using the phia package (De Rosario-Martinez, 2013).

To explore the impact of heterozygosity on fitness related traits we calculated heterozygosity (H_O) as the proportion of heterozygous loci out of the total number of called genotypes for each individual. Introductions could diminish or increase heterozygosity, which in turn could inhibit or stimulate invasion (e.g. inbreeding due to genetic drift or heterosis following admixture). First, we investigated geographical patterns in genetic diversity of populations by testing the effect of range, latitude, their interaction and latitude^2 on population mean H_O. To explore the effect of heterozygosity on selected traits, we included population mean H_O, latitude, their interactions with range and latitude^2 as fixed effects. Both analyses included population STRUCTURE q-values as a random effect. Again, we calculated fixed effects significance with type III Wald F tests, step-wise removing non-significant fixed effects and explored the highest order significant interactions using χ^2 tests with Holm p-value correction with the phia package (De Rosario-Martinez, 2013). To reduce false positive association due to multiple testing, we only tested the response of growth (plant height and biomass) and fitness (total reproductive biomass and average seed weight) related traits. As we identified signatures of repeated local

adaptation in floral sex allocation (Results), we also tested the response of this trait. To reduce false discovery of associations due to the number of traits being tested, we 'fdr' corrected p-values (hereafter q-values) of the combined final models using the p.adjust function, further reducing models when applicable.

RESULTS

Climate niche dynamics

Niche overlap (*D*) was significantly lower than expected between the North American native and Eurasian invasive range ($P<0.001$) although the native and Australasian range did not have significantly lower overlap than expected ($P=0.425$). However, niche stability was low between the native and both invasive ranges ($P<0.001$). Climatic niche unfilling and expansion were not significantly different than the null model, except that especially low expansion was found in the Eurasian population ($P=0.017$), meaning that while the niche shifted, the species did not tend to occupy completely novel climates in its Eurasian range. In both introduced ranges the niche centroid shifted significantly towards hotter, wetter climates ($P<0.001$; Fig. 1). We note that this climate analysis compares the entire Eurasian and Australasian ranges with the native North American range.

Climate of sampled populations

For our common garden analysis we focused only on specific latitudinal transects in Europe and Australia to examine how traits have evolved along latitudinal clines during invasion, and did not include any Asian populations. We include this more general analysis of climate niche to assess how climate shifts might contribute to trait divergence among the ranges generally, although our sampling for the common garden was more limited. Climatic variables across all populations in the native North American, introduced European and introduced Australian ranges could be effectively summarized by the first two principle components (PC) in the PCA (Fig. S2, Table S2, Supplementary Material), which together explained 70.06% of between-population variation. Here, PC1 was strongly associated with latitude, temperature and seasonality, whereas PC2 was mostly precipitation related (Fig S2, Table S2). The climate experienced by Australian populations was distinct from the North American and European ranges (Fig. S2, Table S2), with higher annual, winter and summer temperatures, and with lower seasonal variation. Moreover, given the

sub-tropical location of Australian populations, the sampled populations experienced higher precipitation during the growing season (Fig. S2).

Repeatability in trait clines among ranges

Traits across all North American, European and Australian ranges were well summarized by the PCA, where the first two PCs explained 83.1% of all variation (Fig. S3, Supplementary Material). The main PCs were associated with each trait to a similar extent so no trait syndromes were apparent. Traits measured in Australian plants were generally distinct from the other populations although there was some overlap in multivariate space among the ranges (Fig. S3, Table S3). Multivariate trait analyses revealed a significant two-way interaction between latitude and range ($F_{14,120}$=1.796, p=0.047, Wilks' λ=0.684) (Table S4) suggesting latitudinal trait clines exist, but do not have the same relationship within ranges for all traits. Further dissection of these patterns in univariate analyses revealed maximum growth rate, flowering onset, dichogamy, average seed weight, total reproductive biomass and specific leaf area (SLA) displayed similar latitudinal clines among ranges, indicated by a significant latitude effect but an absence of a higher-order interaction (Fig. 2, Table 2). We identified range differences in latitudinal trait clines for maximum height, total biomass, floral sex allocation and relative reproductive biomass, as indicated by significant range:latitude interactions. However, all of these slopes were significantly different from zero and were in the same direction as the native North American patterns (Fig. 2, Table S5a).

At higher latitudes, plants were shorter, weighed less, reached lower maximum growth rates and flowered earlier. Flowering onset extremes were 14-133 days after transplant (population means for EU20 and AU13, Fig. 2). In all ranges, dichogamy (the temporal separation of pollen dispersal and emergence of receptive stigma within an individual plant) was prevalent. Protogyny (emergence of stigma prior to pollen release) predominated at higher latitudinal populations, with receptive stigmas being visible up to 40 days before any pollen was released within the same plant (EU20)(Fig. 2). Conversely, protandry prevailed at latitudes below 40° from the equator, with pollen release occurring up to 14 days before stigmas became receptive (NC)(Fig. 2). Floral sex allocation followed a similar trend across ranges, with a slight male function bias at lower latitudes, shifting towards an extreme female function bias

at high-latitude populations (Fig. 2). The biggest seeds were found at mid-latitudinal populations (38.6°N in KY), with seeds decreasing in size towards the high and low latitudes (Fig. 2, Table 2). Total reproductive biomass also showed a similar curved relationship, with the combined weight of racemes (male floral sex function) and seeds (female floral sex function) being up to three times as high at mid-latitudinal populations compared to high-latitudinal plants. In contrast, the relative reproductive biomass increased with latitude. Within each range, plants from lower latitudes had lower SLA (Fig. 2).

Trait divergence between ranges

While latitudinal trait clines were repeatable for many traits as described above, we identified shifts in trait values at comparable latitudes as revealed by significant range effects (Table 2). Maximum growth rates were highest in Europe and lowest in Australia (Fig. 2, Table 3a). European plants also flowered later than North American and Australian plants at similar latitudes. The temporal separation between pollen release and the appearance of receptive stigma (dichogamy) was greater in the native North America compared to Europe (Fig. 2, Table 3a). European seeds were heavier and plants had higher total reproductive biomass than those measured in the other ranges. At any given latitude, Australian leaves had higher SLA compared to the native range, with lowest SLA in European populations (Fig. 1, Table 3a).

Dissection of range differences in latitudinal trait clines (maximum plant height, total biomass, floral sex allocation and relative reproductive biomass) revealed most significant interactions between range and latitude were prompted by clinal differences between the introduced European and native North American populations (Table S5, Supplementary Material). For these traits, the discrepancy between North American and European trait values increased with increasing latitudes, such that at high-latitude populations, European plants were taller, heavier and less female-biased in floral sex allocation (Table 3b). Moreover, Australian plants found closest to the equator were significantly shorter than native North American expectations (Table 3b).

Trade-offs between life-history traits

We tested for the presence of a trade-off between plant height and flowering time and investigated associated patterns in dichogamy and floral sex allocation and height. As expected, taller plants started flowering later in both the native and the introduced European range, though this pattern was not significant in introduced Australian populations (Fig. 3, Table 4, Table S6, Supplementary Material). Correspondingly, we observed protogyny and a large female-biased sex allocation in short plants versus protandry with a slight male bias in tall plants. These dichogamy associations with height were not significant in Australia (Fig. 3, Table 4 & S6). However, it is possible that the height of some large Australian plants might have been truncated due to greenhouse conditions.

Associations between heterozygosity and life-history traits

To identify geographic patterns in observed heterozygosity (H_O), we tested the effect of range, latitude and their interaction on H_O. We found no latitudinal patterns in H_O varying within ranges (range:latitude, χ^2_1=3.811, p=0.149) or among all ranges (latitude, χ^2_1=0.000, p=0.986). We did identify variable H_O between ranges (range, (χ^2_1=6.446, p=0.040), resulting from significantly lower H_O in Australia compared to native North America (χ^2_1=6.446; p=0.033). When accounting for latitude and population genetic structure, we found a significant interaction effect between mean population H_O and range on total biomass (Fig. 4, Table 5). Pairwise range comparisons in post-hoc tests revealed a higher H_O that was associated with heavier Australian plants (Table S7, Supplementary Material). Moreover, we found that mean population H_O was positively correlated with seed size in all ranges (Fig. 4, Table 5). We found no effect of individual genomic heterozygosity on plant height, phenology, dichogamy, total or relative reproductive investment and floral sex allocation (Table 5).

DISCUSSION

We show genetically based differentiation along multiple latitudinal clines in all examined traits including plant size, growth, reproductive investment, phenology, dichogamy, SLA, and sex allocation. Remarkably, the clinal patterns apparent in the native range evolved repeatedly within both introduced ranges over the course of only 100-150 years and despite limited neutral genetic variation in the introduced Australian range. These patterns are consistent with rapid adaptation, as we accounted

for neutral genetic differentiation. Moreover, low effects of maternal environment are expected (Hodgins & Rieseberg, 2011) and the introduction history of this species (van Boheemen *et al.*, 2017) reveals climate-matching (Maron *et al.*, 2004) is unlikely. The adaptive trait divergence at similar latitudes, together with a centroid shift to warmer and wetter climates in the introduced ranges, could suggest invasive populations have adapted to more productive environments following introduction. The observed rapid evolution has implications for the evolutionary potential of this species and further range expansion following climate change. Furthermore, the divergence of reproductive traits such as flowering time, sex allocation and seed size during recent range expansion should impact the production of allergenic pollen as well as the abundance and dispersal of seed that could impact spread.

Climate niche shifts

Higher resource levels, such as increased water availability, are a known contributor to invasion in many plant species (Blumenthal, 2006; Dlugosch *et al.*, 2015b). Increased resource availability may occur through a shift in the fundamental or realized niche during invasion. The latter can result from reductions in competition, perhaps reflecting the presence of a vacant niche in the introduced range (e.g. Dlugosch *et al.*, 2015b). Climate niche dynamics analysis reveals higher *A. artemisiifolia* abundance in warmer and wetter climates in the introduced ranges compared to the native range. It is possible that this centroid shift reflects an historic effect where colonization of warmer and wetter environments occurred earlier, or perhaps by genotypes pre-adapted to these climates (*but see* van Boheemen *et al.*, 2017). Alternatively, the shift might reflect changes in biotic interactions leading to greater abundance of this species in high resource environments or differences in the availability of these climates in the introduced regions. Evolutionary processes that allow introduced species to colonize warmer and wetter environments than those occupied by native plants could also cause centroid shifts. This evolutionary interpretation is supported as Australian populations follow trait trajectories parallel to, but extending beyond, those of the native range.

Repeated latitudinal clines

Our common garden experiments using samples collected across multiple similar latitudinal gradients, reveal that local adaptation can happen quickly and

predictably, with repeated evolution of native clines in both of the introduced ranges. Latitudinal clines in phenology and size are a common feature of many geographically widespread plant species (e.g. Colautti *et al.*, 2010; Li *et al.*, 2014), with *A. artemisiifolia* flowering shown to be driven by the association between season length and latitude (Ziska *et al.*, 2011). Short season lengths at high latitudes can select for earlier flowering (Bradshaw & Holzapfel, 2008; Colautti & Barrett, 2013), while the evolution of delayed flowering at low latitudes reflects the trade-off between size and the timing of reproductive maturity, where fitness is maximized by flowering later at a large size (Colautti *et al.*, 2010; Colautti & Barrett, 2013). This correlation between plant size and flowering time has been reported for *A. artemisiifolia* (Hodgins & Rieseberg, 2011; Leiblein-Wild & Tackenberg, 2014; Scalone *et al.*, 2016) and our results are consistent with rapid genetic differentiation in plant size, growth rates and phenology in response to latitude-associated selection pressures such as season length.

We exposed repeated patterns of genetic differentiation in sex allocation strategy over similar latitudinal clines, consistent with rapid adaptation to local climate. Plants sourced from higher latitudes displayed female-biased sex-allocation and protogyny, with more balanced floral sex allocation and a decrease in the temporal separation of male and female function towards the equator. Previous studies on *A. artemisiifolia* showed plasticity for sex-allocation and dichogamy in relation to plant size (Paquin & Aarssen, 2004; Friedman & Barrett, 2009; Friedman & Barrett, 2011) and ample genetic variation for evolution to act on (Friedman & Barrett, 2011). Local seed dispersal should lead to saturating female gain curves (Lloyd & Bawa, 1984; Sakai & Sakai, 2003), yet more linear male function gain curves are predicted in wind pollinated plants with increasing height (Klinkhamer *et al.*, 1997; Friedman & Barrett, 2009). As a result, outcrossing wind-pollinated hermaphrodites with local seed dispersal, such as *A. artemisiifolia*, are predicted to adaptively change sex allocation to be more male-biased with increase in size (Lloyd, 1984; De Jong & Klinkhamer, 1989; de Jong & Klinkhamer, 1994; Klinkhamer *et al.*, 1997), consistent with patterns observed in this study. Spatial heterogeneity has been observed in animal pollinated plants, where small, resource limited individuals often allocate more resources to male function (Korpelainen, 1998; Guo *et al.*, 2010). However, these studies are on wild populations and cannot separate environmental and genetic effects in allocation patterns along resource gradients. Our current findings from common

garden experiments are therefore novel in identifying genetic differentiation among populations in sex allocation over spatial gradients.

Trait divergence among the ranges

Many hypotheses aim to explain the success of invasive species, including the evolution of increased competitive ability (EICA) through escape from native herbivores (Blossey & Notzold, 1995). Moreover, if trade-offs between performance and abiotic stress tolerance occur, greater resources could facilitate the evolution of more competitive phenotypes in introduced ranges (Grime, 1977; Bossdorf *et al.*, 2005; He *et al.*, 2010; Dlugosch *et al.*, 2015b). Our reported trait shifts in European population compared to natives at equivalent latitudes indeed suggest an increase in competitive ability, commonly measured as elevated plant growth and reproductive effort (Felker□Quinn *et al.*, 2013). These observations might reflect the warmer and wetter European climate (Fig. 1), as no general evidence for EICA has been found in Europe (Hodgins & Rieseberg, 2011; van Boheemen *et al.*, 2018) despite shifts in herbivore community composition in Europe and Australia (Genton *et al.*, 2005; Palmer & McFadyen, 2012; Essl *et al.*, 2015). Notably, although Europe was identified as the introduction source for Australian populations (van Boheemen *et al.*, 2017), traits measured within each range were highly dissimilar. Most of the sampled Australian populations extended beyond absolute latitudes of the other populations and were situated in warmer, less seasonal climates (Fig. S2). These factors might explain Australian trait variation beyond values observed in source populations.

Heterozygosity and invasion

Genetic drift within small founding populations and on the invasion front can lead to reduced genetic diversity, potentially impacting additive genetic variation (Dlugosch & Parker, 2008a; Excoffier & Ray, 2008; Peischl *et al.*, 2013; Bock *et al.*, 2015). In *A. artemisiifolia*, Australian populations were bottlenecked and likely subjected to high genetic drift, whereas multiple introductions into Europe from distinct native sources has implicated admixture as a driver of invasion success (van Boheemen *et al.*, 2017). We found the biomass of Australian, but not European, plants was indeed associated with heterozygosity, providing only partial support for the fitness benefits of heterozygosity during invasion (Peischl & Excoffier, 2015). Admixture and heterosis are unlikely to be main drivers of invasiveness in Europe, as

we found no relationship between population level heterozygosity and any trait other than seed size. Indeed, in most experimentally admixed European and native *A. artemisiifolia* crosses heterosis was absent (Hahn & Rieseberg, 2017). These observations suggest demographic processes had very limited consequences (negative or positive) on the fitness of these introduced populations. However, local adaptation of life history traits such as plant size across broad environmental gradients may mask heterozygosity-fitness correlations. Future studies could address this question by examining the association between heterozygosity and fitness in single populations (e.g. Conte *et al.*, 2017).

In plants, reduced seed size is one trait that could aid dispersal and might therefore be expected to evolve during range expansion (Bartle *et al.*, 2013; Huang *et al.*, 2015). Spatial sorting for dispersal traits at the expansion front has been well documented in other invasions, such as the cane toads (Estoup *et al.*, 2004; Phillips *et al.*, 2006). In Europe, spatial sorting for increased dispersal, and therefore smaller seeds, could have occurred at the range edge during expansion northwards. However, this mechanism would only explain the seed size decline in low-latitudinal populations in Australia, where range expansion likely occurred both north- and southward (Palmer & McFadyen, 2012; van Boheemen *et al.*, 2017). Moreover, recent evidence shows dispersal distance is determined to a much larger extent by plant height than seed traits (Thomson *et al.*, 2011; Tamme *et al.*, 2014; Augspurger *et al.*, 2017). The association between seed size and mean population heterozygosity we identified in all three ranges could be expected when small seeds aid dispersal, as founder effects should also reduce heterozygosity during colonization. Though additional factors likely shape seed size evolution, our findings suggest seed size divergence could represent an important difference in life-history strategies between ranges. Moreover, we observed patterns indicating a relationship between genomic and ecological dynamics potentially linked to range expansion and colonization.

Conclusion

Invasive species often exhibit rapid adaptation despite facing novel selective pressures (Lachmuth *et al.*, 2011; Colautti & Barrett, 2013; Chown *et al.*, 2014; Turner *et al.*, 2014). Moreover, the success of invasives is considered paradoxical as strong demographic changes are predicted to enhance inbreeding and reduce genetic variation and, consequently, evolutionary potential (Allendorf & Lundquist, 2003).

We investigated these apparent contradictions in a comprehensive study. We compared the native range with multiple introduced ranges with highly distinct demographic histories, characterized similarities and shifts in climatic niches, tested adaptation in a large number of life-history traits and explored heterozygosity-fitness associations while accounting for non-adaptive population differentiation. We found strong evidence for parallel adaptation in all three ranges. This study therefore emphasizes that although introduction dynamics can affect genetic diversity (Dlugosch & Parker, 2008a) the adaptive potential of those traits might not be constrained to a similar extent (Dlugosch *et al*., 2015a).

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AUTHOR CONTRIBUTIONS

LAB and KH designed the project, with data collection and analyses carried out by LAB and refined by KH. DZA developed and carried out the niche distribution modelling. All authors discussed the results and contributed to the MS writing.

DATA ACCESSBILITY

Sequence data are available at the National Center for Biotechnology Information **(**NCBI) Sequence Read Archive under Bioproject PRJNA449949.

Table 1. Traits included in this study.

Trait	Description
Max. height	Maximum measured height
Total biomass	Above- and belowground dry biomass
Max. growth rate	Sensu Chuine *et al.* (2001)
Flowering onset	First recorded day of flowering (number of days after second transplant); first day of pollen release (male function) or receptive female function
Dichogamy	First recorded day of pollen release - first recorded day of receptive female function (a positive value is protogyny, a negative value is protandry)
Floral sex allocation (female/male)	Female (seeds) / male (raceme) dry weight (a value >1 is higher biomass allocation to female function)
Weight per seed	Dry weight per seed in milligrams, averaged over 20 seeds (where available)
Total reproductive biomass	Female (seeds) and male (raceme) dry weight
Relative reproductive biomass	Total reproductive biomass / total plant biomass
Specific leaf area	Leaf area of fully expanded fresh leaf/leaf dry weight

Table 2. *Ambrosia artemisiifolia* population mean trait responses to range, latitude, their interaction and latitude^2, with population q-values as random effects. We reported Wald type III F-values, with Kenward-Roger degrees of freedom as subscript and symbols specifying significance (fdr corrected q) of effect.

	Range	Latitude	Latitude^2	Range:Latitude
Max. height	$3.886_{2,70.57}$*	$55.963_{1,71.9}$***	$0.581_{1,70.97}$(ns)	$4.068_{2,70.35}$*
Total biomass	$3.344_{2,69.25}$#	$3.227_{1,70.98}$#	$5.214_{1,70.97}$*	$3.667_{2,69.14}$*
Max. growth rate	$6.405_{2,71.98}$**	$21.658_{1,70.76}$***	$2.025_{1,73}$(ns)	$1.101_{2,69.14}$(ns)
Flowering onset	$6.862_{2,71.978}$**	$158.301_{1,70.763}$***	$1.102_{1,73}$(ns)	$3.43_{2,69.14}$#
Dichogamy	$10.521_{1,73}$**	$8.163_{2,71.18}$**	$6.805_{1,72.99}$*	$3.157_{2,69.14}$#
Floral sex allocation (female/male)	$6.354_{2,69.82}$**	$6.048_{1,66.76}$*	$7.949_{1,66.27}$*	$7.094_{2,69.77}$**
Weight per seed	$4.156_{2,66.13}$*	$25.758_{1,72.41}$***	$23.704_{1,72.88}$***	$0.18_{2,67.29}$(ns)
Total reproductive biomass	$27.478_{1,73}$***	$6.856_{2,71.18}$**	$20.509_{1,72.99}$***	$3.427_{2,69.14}$#
Relative reproductive biomass	$6.436_{2,70.57}$**	$22.839_{1,71.9}$***	$2.997_{1,70.97}$(ns)	$6.422_{2,70.35}$**
Specific leaf area	$7.626_{2,67.39}$**	$17.167_{1,66.45}$***	$1.807_{1,63.65}$(ns)	$3.003_{2,65.14}$#

ns q>0.1; # q<0.1; * q<0.05, ** q<0.01; *** q<0.001

Table 3. Range differences of *A. artemisiifolia* population mean traits at comparable latitudes for significant (q<0.05) range effects (a, Table 2) and trait differences between ranges at minimum (min) and maximum (max) latitudes (Figure S4, Supplementary Material) for significant range:latitude interactions by comparing trait values at (b, Table 2).

a)	**North America - Europe**	**North America - Australia**	**Europe - Australia**
Max. growth rate	12.328_1**	4.264_1*	1.278_1**
Flowering onset	16.465_1***	0.123_1(ns)	13.269_1***
Dichogamy	18.8_1***	0.077_1(ns)	4.87_1#
Weight per seed	7.44_1*	2.059_1(ns)	6.002_1*
Total reproductive biomass	15.228_1***	0.811_1(ns)	6.516_1*
Specific leaf area	2.734_1#	14.936_1***	14.736_1***

)	**North America-Europe**	**North America-Australia**	**Europe-Australia**
Max. growth rate	13.269_1***	4.178_1*	11.606_1**
Flowering onset	16.465_1***	0.123_1(ns)	13.269_1***
Dichogamy	18.8_1***	0.077_1(ns)	4.87_1#
Weight per seed	7.44_1*	2.059_1(ns)	6.002_1*
Total reproductive biomass	15.228_1***	0.811_1(ns)	6.516_1*
Specific leaf area	2.734_1#	14.936_1***	14.736_1***

b)	**North America (NA) - Europe (EU)**		**North America (NA) - Australia (AU)**	
Trait	**EU_{min}**	**NA_{max}**	**NA_{min}**	**AU_{max}**
Max. height	8.679_1**	25.236_1***	4.818_1#	0.039_1(ns)
Total biomass	0.339_1(ns)	15.572_1***	0.564_1(ns)	1.307_1(ns)
Floral sex allocation (female/male)	0.012_1(ns)	26.417_1***	1.586_1(ns)	0.096_1(ns)
Relative reproductive biomass	4.289_1(ns)	7.557_1*	0.065_1(ns)	0.017_1(ns)

ns q>0.1; # q<0.1; * q<0.05, ** q<0.01; *** q<0.001

Table 4. Flowering time and sex function allocation responses to maximum plant height (Height), range and their interaction, with individual q-values and population as random effects. We reported Wald type III F-values test, Kenward-Roger degrees of freedom as subscript and symbols specifying significance of effect.

Trait	Range	Height	Range:Height
Flowering onset	$20.658_{2,606.14}$***	$105.082_{1,766.54}$***	$14.767_{2,671.02}$***
Dichogamy	$6.503_{,510.44}$**	$51.235_{1,670.96}$***	$10.621_{2,545.18}$***
Floral sex allocation (female/male)	$17.451_{2,508.47}$***	$138.084_{1,659.31}$***	$16.963_{2,508.47}$***

ns q>0.1; # q<0.1; * q<0.05, ** q<0.01; *** q<0.001

Table 5. Trait responses (population mean) to range, latitude, latitude^2, heterozygosity (H_O) and interactions, with population q-values as random effects. We reported Wald type III F-values test values, Kenward-Roger degrees of freedom as subscript and symbols specifying significance of effect.

	H_O	**Range**	**Latitude**	**Latitude^2**	**H_O:Range**	**Range:Latitude**
Max. height	$0.007_{1,70.75}$(ns)	$3.435_{2,69.52}$#	$51.007_{1,70.95}$***	$0.198_{1,69.96}$(ns)	$0.095_{2,59.46}$(ns)	$3.699_{2,69.23}$#
Total biomass	**$8.455_{1,53.099}$***	$4.942_{2,65.157}$*	$108.362_{1,68.316}$***	$0.699_{1,69.812}$(ns)	**$3.947_{2,64.456}$***	$2.055_{2,66.152}$(ns)
Total reproductive biomass	$0.263_{1,71.8}$(ns)	$7.123_{2,70.12}$**	$19.116_{1,71.91}$***	$24.912_{1,71.96}$***	$0.274_{2,59.46}$(ns)	$1.721_{2,68.2}$(ns)
Weight per seed	**$5.858_{1,38.45}$***	$3.271_{2,47.3}$#	$23.516_{1,71.93}$***	$20.776_{1,71.84}$***	$0.069_{2,25.95}$(ns)	$0.332_{2,55.71}$(ns)
Floral sex allocation (female/male)	$0.12_{1,69.63}$(ns)	$6.619_{2,68.56}$**	$6.229_{1,69.87}$*	$7.55_{1,69.84}$*	$0.096_{2,59.46}$(ns)	$7.287_{2,68.47}$**

ns q>0.1; # q<0.1; * q<0.05, ** q<0.01; *** q<0.001

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THE FOLLOWING SUPPORTING INFORMATION IS AVAILABLE FOR THIS ARTICLE:

Fig. S1 Sampling locations

Fig. S2 Principle component analysis of climatic and geographic variables

Fig. S3 Principle component analysis of traits

Fig. S4 Graphical representation of performed trait comparisons at range edges

Table S1 List of climatic and geographic variables

Table S2 Correlation and principle component loadings of climatic and geographic variables

Table S3 Trait correlation and principle component loadings

Table S4 MANCOVA testing the effect of range treatment and latitude on selected traits

Table S5 Trait-latitude associations within ranges and range differences

Table S6 Flowering onset, dichogamy and floral sex allocation associations with height within ranges and range differences

Table S7 Range differences of heterozygosity-biomass associations

Methods S1 Detailed methods for common garden set-up and data collection

Notes S1 Australian trait divergence

FIGURE LEGENDS

Figure 1. Climatic niche models of the native North American population (A) and introduced Australasian (B) and Eurasian (C) populations. Each panel shows the climate space occupied in the respective range, with a PCA variable corresponding to temperature on the x-axis and a PCA variable corresponding to precipitation on the y-axis. Color indicates occurrence probability in a given climate (red: high occurrence, green: medium occurrence, blue: low occurrence, grey: no occurrence). Open circles plot climates of the North American source localities. Closed circles plot the climates of the respective introduced range. On panels B and C, the dashed line encloses the climates shared by both the native North American and respective introduced range.

Figure 2. Traits responses (population means) to absolute latitude in the native North American (blue triangles), introduced European (green squares) and Australian (red circles) ranges, with model predictions and 95% shaded confidence intervals from step-wise reduced models (Table 2).

Figure 3. Flowering time, dichogamy and floral sex allocation responses to maximum plant height (individual values) in the native North American (NA, blue triangles), introduced European (EU, green squares) and Australian (AU, red circles) ranges, with model predictions and 95% shaded confidence intervals from step-wise reduced models (Table 4). Differences in slopes are indicated by letters and are significantly different from zero unless otherwise indicated (ns)(Table S5, supporting information).

Figure 4. Total biomass and weight per seed response to heterozygosity (population means) in the native North American (blue triangles), introduced European (green squares) and Australian (red circles) ranges, with model predictions and 95% shaded confidence intervals from step-wise reduced models (Table 5). Slopes of predicted lines are significantly different from zero, unless otherwise indicated as (ns).

Fig. 1

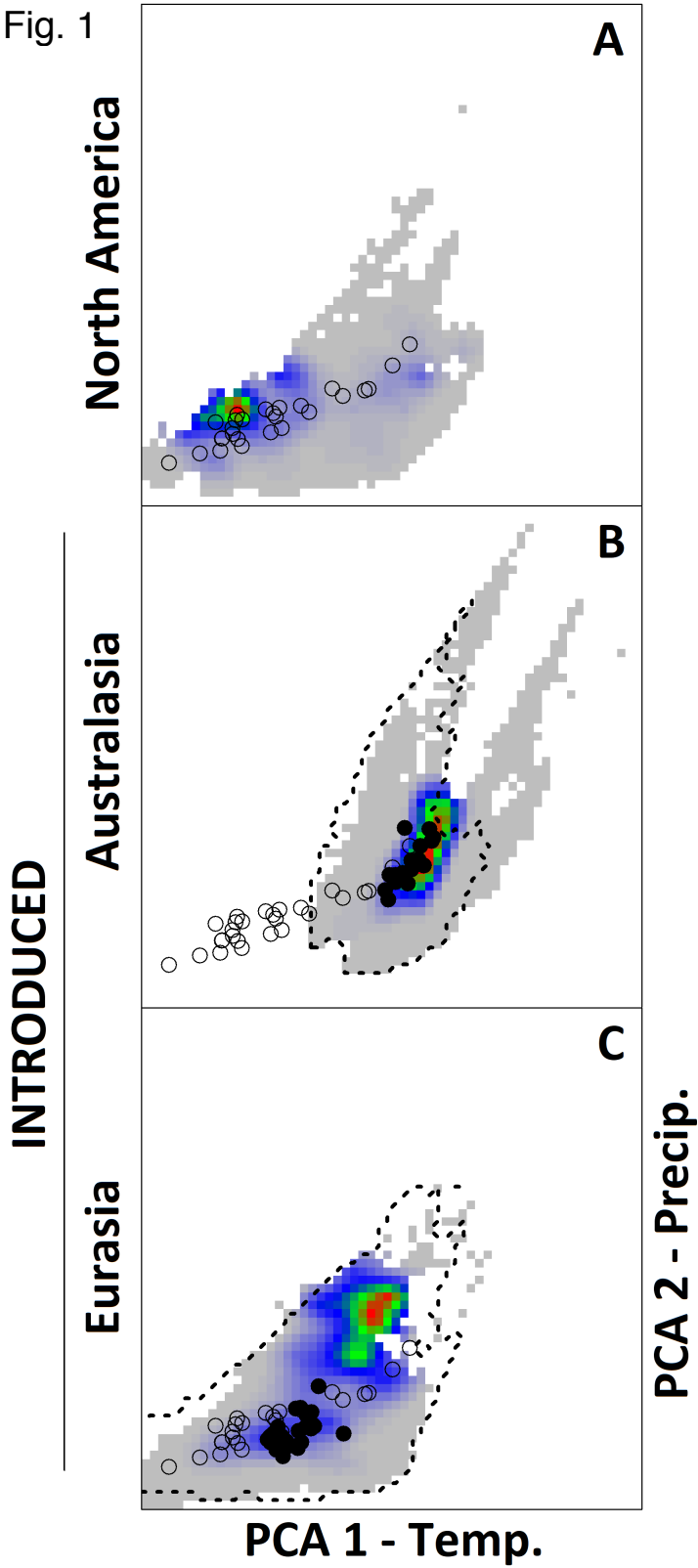

A
B
C
North America
Australasia
Eurasia
INTRODUCED
PCA 2 - Precip.
PCA 1 - Temp.

Fig. 2

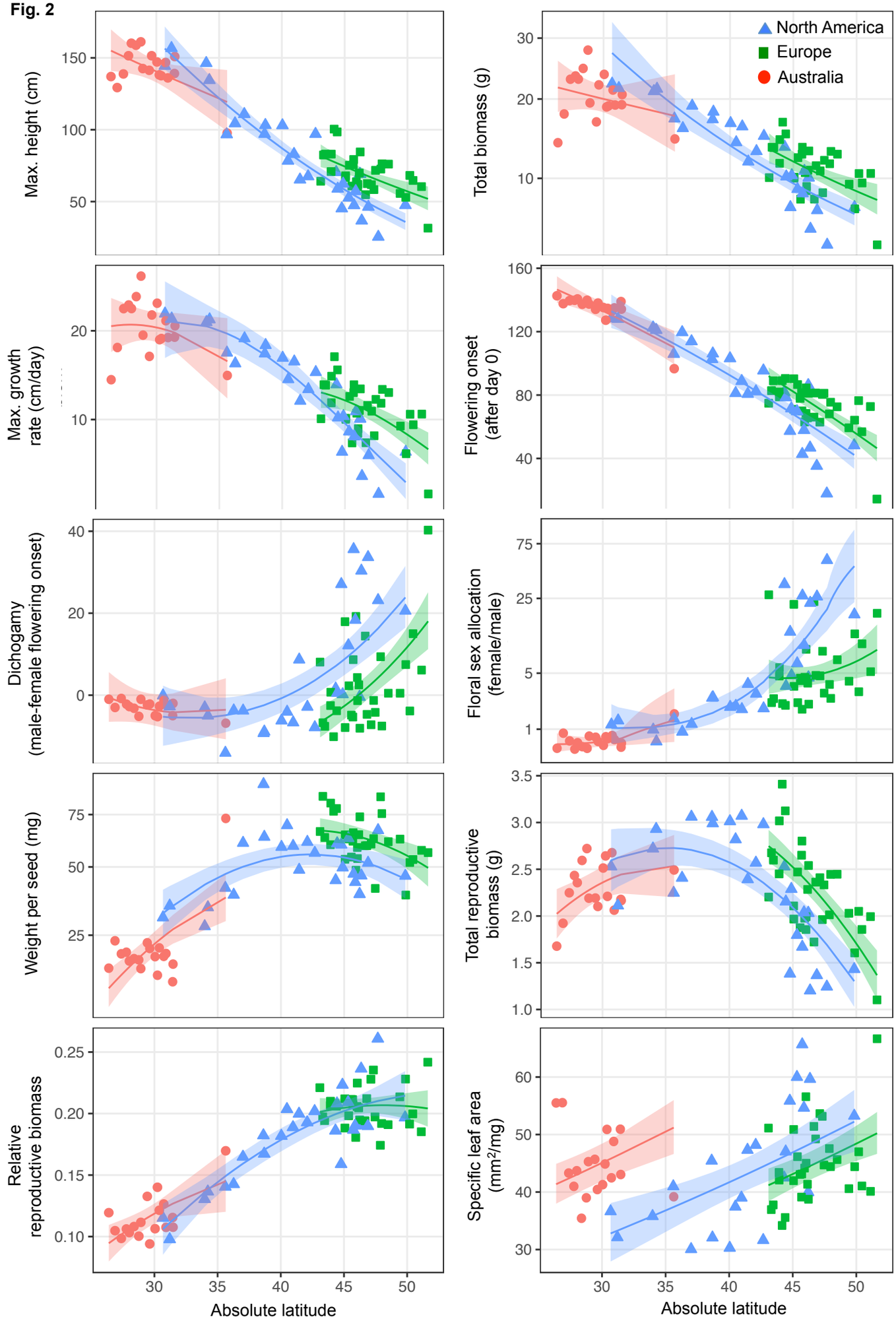

North America
Europe
Australia
Max. height (cm)
Total biomass (g)
Max. growth rate (cm/day)
Flowering onset (after day 0)
Dichogamy (male-female flowering onset)
Floral sex allocation (female/male)
Weight per seed (mg)
Total reproductive biomass (g)
Relative reproductive biomass
Specific leaf area (mm²/mg)
Absolute latitude
Absolute latitude

Fig. 3

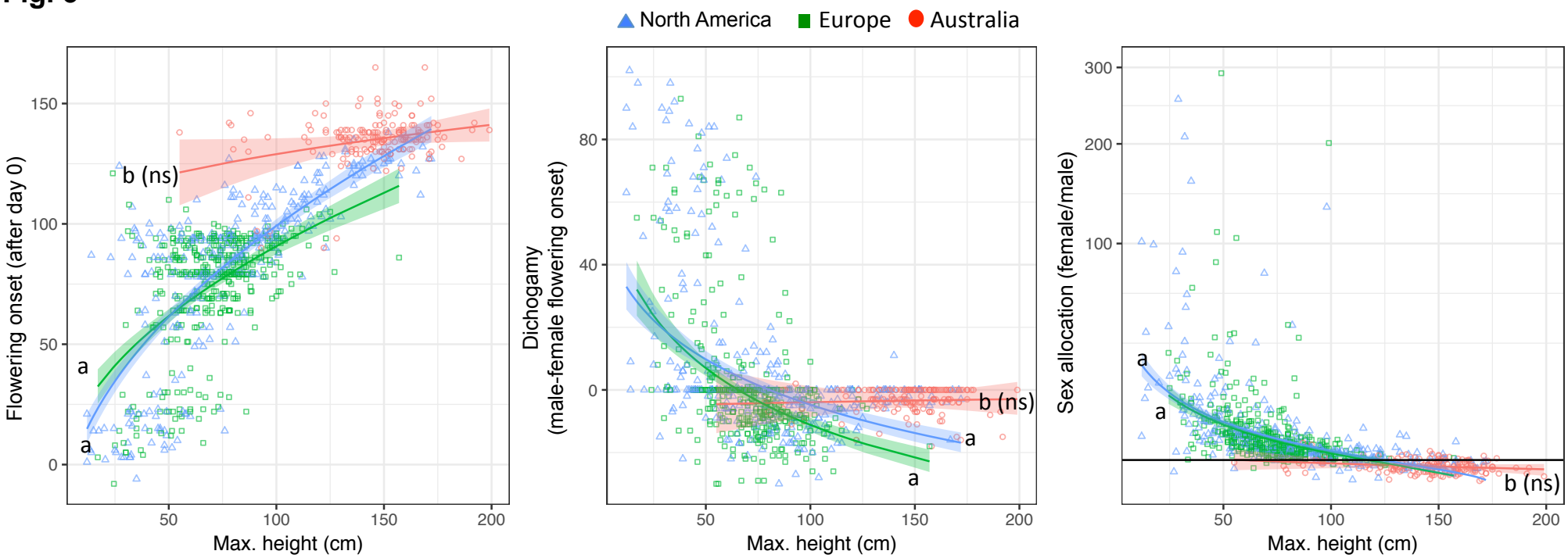
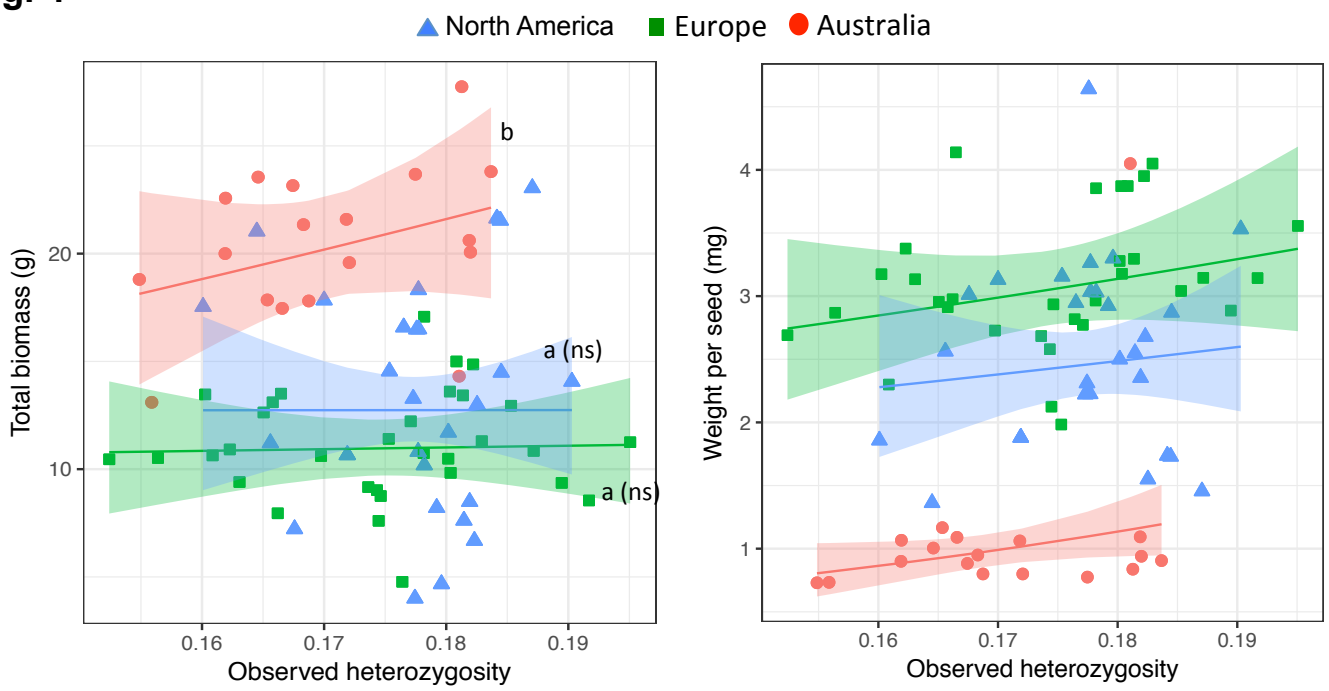

North America
Europe
Australia
Flowering onset (after day 0)
Max. height (cm)
b (ns)
a
a
Dichogamy
(male-female flowering onset)
Max. height (cm)
b (ns)
a
a
Sex allocation (female/male)
Max. height (cm)
a
a
b (ns)

Fig. 4


North America
Europe
Australia
b
a (ns)
a (ns)
Total biomass (g)
20
10
0.16
0.17
0.18
0.19
Observed heterozygosity
Weight per seed (mg)
4
3
2
1
0.16
0.17
0.18
0.19
Observed heterozygosity