RESEARCH ARTICLE

Experimental admixture among geographically disjunct populations of an invasive plant yields a global mosaic of reproductive incompatibility and heterosis

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Abstract

1. Invasive species have the ability to rapidly adapt in the new regions where they are introduced. Classic evolutionary theory predicts that the accumulation of genetic differences over time in allopatric isolation may lead to reproductive incompatibilities resulting in decreases in reproductive success and, eventually, to speciation. However, experimental evidence for this theoretical prediction in the context of invasive species is lacking. We aimed to test for the potential of allopatry to determine reproductive success of invasive plants, by experimentally admixing genotypes from six different native and non-native regions of *Centaurea solstitialis*, an invasive forb for which preliminary studies have detected some degree of reproductive isolation between one native and non-native region.

2. We grew plants under common garden conditions and outcrossed individuals originating from different source populations in the native and introduced range to evaluate reproductive success in terms of seed to ovule ratio produced. We also assessed geographical and genetic isolation among *C. solstitialis* regions as a potential driving factor of reproductive success.

3. Experimental admixture generated mixed fitness effects, including significant increases, decreases and no differences in reproductive success as compared to crosses within population (control). *Centaurea solstitialis* invasive populations in the Americas generated preponderantly negative fitness interactions, regardless

of the pollen source, suggesting selection against immigrants and reinforcement. Other non-native populations (Australia) as well as individuals from the native range of Spain demonstrated an increase in fitness for between-region crosses, indicating inbreeding. These differences show an asymmetrical response to inter-regional gene flow, but no evidence of isolation by distance.

4. *Synthesis*. The speed of adaptation and the accumulation of reproductive incompatibilities among allopatric populations of invasive species might be more rapid than previously assumed. Our data show a global mosaic of reproductive outputs, showcasing an array of evolutionary processes unfolding during colonization at large biogeographical scales.

1 | INTRODUCTION

Invasive species provide insight into evolutionary processes such as reproductive isolation and allopatric speciation because they are very recently distributed over distant and often isolated geographical regions in different parts of the world and show remarkable adaptive potential (Callaway & Maron, 2006; Hille Ris Lambers et al., 2013; Lavergne & Molofsky, 2007; Maron et al., 2004; Rosche et al., 2019). When an ancestral population of a species becomes divided into two or more geographically distinct subpopulations either through vicariance or dispersal, these subpopulations may start to diverge and become genetically and reproductively isolated from the ancestral population, potentially leading to the formation of new species (Coyne & Orr, 2004). Assuming these populations are brought back into contact, their potential to exchange genetic material with the parent population can be reduced by incipient reproductive barriers (Lowry et al., 2008; Montesinos et al., 2012; Nosil, 2012). Admixture between these isolated lineages may result in different fitness consequences, depending on the population's historical, ecological and evolutionary background (Ellstrand & Schierenbeck, 2000; Verhoeven et al., 2011). During colonization of new environments, introduced populations of a species often experience reduced genetic diversity due to founder effects and do not yet show patterns of local adaptation (Verhoeven et al., 2011). Under this scenario, admixture is expected to be beneficial by masking deleterious alleles, generating new genotypes, increasing standing genetic variation and releasing inbreeding load (Ellstrand & Schierenbeck, 2000). However, in native populations that are locally adapted, admixture is mostly selected against (but may occur during range expansion) because it disrupts the co-adapted gene complexes, leading to genetic incompatibilities in recombinant individuals (Hopkins, 2013; Verhoeven et al., 2011). Characterizing the patterns of intraspecific variation in reproductive isolation in

different traits can provide insights into the evolutionary processes that underly the early stages of speciation (Barnard-Kubow & Galloway, 2017). There are multiple factors that can restrict gene flow among populations which can be grouped into intrinsic (genetic incompatibilities) and extrinsic (reduced hybrid fitness in parental environments), acting at different life stages such as pre-zygotic or post-zygotic stages (Coyne & Orr, 2004; Rundle & Nosil, 2005). In addition, gene flow can also be reduced because of species' limited dispersal abilities showing patterns of isolation with distance (IBD, Wright, 1943), or because of environmental heterogeneity among populations. The expectation is that the degree of isolation will be larger between more distant populations. Extensive reviews of barriers to gene flow describe these processes (Baack et al., 2015; Hendry et al., 2007).

Documenting the rates at which reproductive isolation develops in allopatry as well as its effects on different fitness components can be done by means of experimental crosses between previously isolated lineages (Sapir & Mazzucco, 2015; Verrell & Arnold, 1989; Waser & Price, 1989). Relatively few studies have evaluated the potential consequences of admixture across invasive species ranges (Barker et al., 2019; Dlugosch, Anderson, et al., 2015; Montesinos et al., 2012; Shi et al., 2018; van Kleunen et al., 2015; Wolfe et al., 2007). For example, van Kleunen et al., (2015) observed an increase in biomass and seed production in F_1 hybrids of *Mimulus guttatus*, when outcrossing plants from native and invasive populations, with fitness benefits partially maintained in the F_2 generation (Li et al., 2017). Likewise, admixture during invasion of North America by the European weed *Silene vulgaris* increased the fitness of recombinant genotypes by more than twofold in terms of fruit production, compared to less admixed genotypes (Keller & Taylor, 2010). Others found evidence of reproductive barriers in seed formation between non-native populations of *Centaurea solstitialis* in the United States and native Spain (Montesinos et al., 2012). Interestingly, an F_1 generation of

progenies of *C. solstitialis* derived from crosses among geographically distant populations in the native range, most commonly exhibited increased growth rates, consistent with heterosis (Barker et al., 2019). Admixture experiments are thus a powerful tool to enhance our understanding of invasive species dynamics, such as underlying mechanisms of reproductive isolation and the rate at which they occur.

Here, we test the hypothesis that local adaptation recently developed through allopatry will result in rapid divergence in reproductive success for crosses between geographically disjunct populations of *C. solstitialis*. The species is native to Eurasia and was introduced to Australia and the Americas approximately 200 years ago, where it established as an aggressive invader (DiTomaso et al., 2006). In its short time since introduction, *C. solstitialis* had evolved strong phenotypic divergence in numerous ecological traits between native and non-native ranges, including divergence in seed mass, germination speed, density of individuals in the field, plant size (Graebner et al., 2012; Hierro et al., 2009, 2013, 2020; Uygur et al., 2004), seed starch content (Widmer et al., 2007), leaf defence (Sotes et al., 2015), flowering phenology and competitive ability (Montesinos & Callaway, 2017). Moreover, there is evidence for incipient reproductive incompatibilities between populations in some neo-allopatric and native regions (Montesinos et al., 2012). In spite of such phenotypic differences, native and non-native populations do not display notable differentiation in genetic makeup (Barker et al., 2017; Eriksen et al., 2014), and successful crosses between some geographically distant populations have been generated (Barker et al., 2019; Montesinos et al., 2012). In this study, we addressed the following predictions: (a) experimental admixture within the native range will lead to increased fecundity indicating inbreeding depression; (b) admixture will result in decreased fecundity in the introduced range, because populations in those regions are already the product of several past admixture events and (c) reproductive output will decrease with increasing the crossing distance, as a result of isolation by distance.

2 | MATERIALS AND METHODS

2.1 | Study species

Centaurea solstitialis (Asteraceae) is a globally distributed herbaceous annual plant species, diploid throughout its range (Irimia et al., 2017), and associated with human disturbance. The species has a branched appearance with reproductive structures found at the end of each branch. Male and female stages are spatially and temporally separated and the species is predominantly self-incompatible (Maddox et al., 1996), relying on outcrossing (i.e. pollinators) to set seeds, although sporadic self-fertilization may occur too (Petanidou et al., 2012; Sun & Ritland, 1998). The flower is a capitulum consisting of 20–80 single florets, that have a sequential development (Leong et al., 2014; Maddox et al., 1996). *Centaurea solstitialis* has a late reproductive phenology compared to associated vegetation communities, starting to flower from end of May to senescence or frost (Roché et al., 1997). Flower heads produce two types of seeds (achenes), one with a short plume (pappus) and the other lacking that plume (Hierro et al., 2009; Maddox et al., 1996).

2.2 | Pollination experiment

Mature seeds of *C. solstitialis* were collected in the field between 2009 and 2014, from 50 natural populations spanning localities in the native Turkey (regarded by many authors as the centre of origin of the species; Eriksen et al., 2014) and Spain (the main source of seeds that have colonized the Americas; Barker et al., 2017) and important introductions in the non-native regions of (a) central Argentina where *C. solstitialis* is highly invasive, (b) central Chile, where the species has a sparse and localized distribution and (c) California coast (highly invasive), and southeastern Australia (sparse distribution). Mother plants were sampled at least 3 m from each other during field surveys, and across representative transects of

FIGURE 1 Map of *Centaurea solstitialis* site collection in the native and non-native range (global view and insets on each region) with the number of individuals from each region that were used in the pollination experiment given in parenthesis. Labels correspond to geographical regions where *C. solstitialis* populations were sampled namely: (a) California, (b) South America (Chile and Argentina), (c) Spain, (d) Turkey, (e) Australia
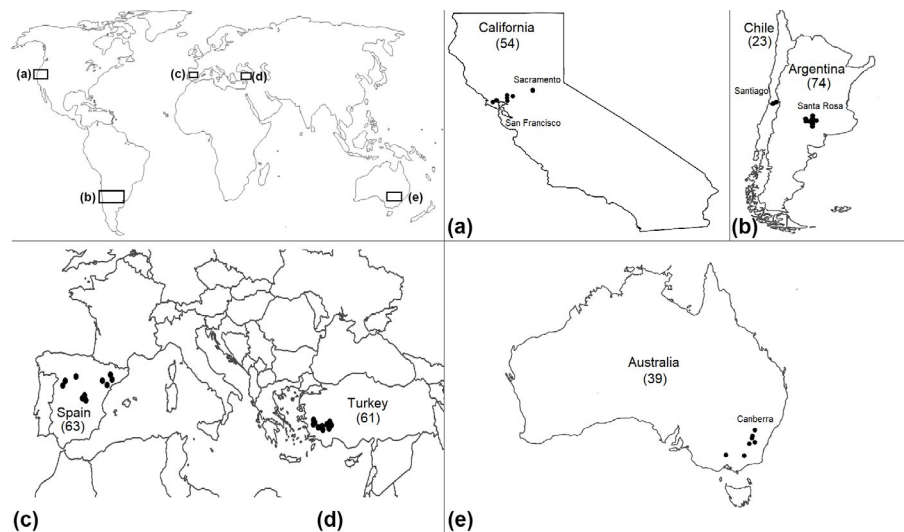
the population to avoid clumping and edge effects. Populations were spread across the distribution of the species in each region, in order to be representative of each country sampled (see Figure 1 for a map of collection sites).

Seeds originating from up to 10 different maternal individuals per population (N = 314, Table S1, Supporting Information) were germinated in spring 2017 and grown to senescence in 2L pots filled with commercial soil (Substratos Profissionais, Leal & Soares S.A., Portugal), in a pollinator-excluded glasshouse. Plants were watered daily and chemical fertilizer was supplied three times during the experiment, before the flowering onset (Fertiberia Jardin, Spain, NPK: 8-4-6 plus microelements). Plants were randomly rotated within the greenhouse monthly to minimize variation in microclimatic effects. We sprayed the plants twice to stop fungal infection, each before the onset of the anthesis, with Tebuconazol (Luna Experience, Bayer, containing 200 g/L or 17.7% p/p fluopyram and 200 g/L or 17.7% p/p tebuconazol). Controlled manual cross-pollinations were made by rubbing mature capitula (flower heads) to each other. Maturity was assessed based on the presence and abundance of pollen and on the receptiveness of stigmas, and preliminary tests across the flower anthesis developed during previous years were used to determine the optimal phenological stage for manual cross-pollination. Experimental crosses began when approximately 10% of the total flower heads initiated flowering and were conducted from mid-June to the end of September 2017, which corresponds to the natural reproductive phenology observed in the field. Only one pollen donor was used on each individual capitula. Treated capitula were grown until ripe and harvested for ovule and seed counting. Five different pollination treatments were applied to each individual plant: (a) spontaneous self-pollination; (b) manual self-pollination); (c) manual within-population pollination (crosses between two different individuals of the same population); (d) manual between-populations within-region pollination (crosses between two individuals from different populations within the same region) and (5) manual between-region pollination (crosses between two individuals from two different randomly selected populations in two different world regions). All individual plants (314) used in this experiment acted as both pollen donors and pollen recipients. In total, we performed 30 different between-region cross combinations and six within-region crosses on 314 different maternal plants. Each of the six types of pollination treatments were extensively replicated at the individual mother plant and maternal population (see excel file available from Dryad Digital Repository; https://doi.org/10.5061/dryad.t76hdr80d, for an overview of the extent of cross replication) (Irimia et al., 2021).

2.3 | Fruit harvesting and seed counting

Individual capitula were collected when ripe, approximately 2 weeks after pollination. Flower heads were tagged, stored in paper bags and dissected in the laboratory to count the total number of ovules with pappus and ovules without pappus in a capitulum, and the number of viable and non-viable seeds present in each capitulum. Seed

viability was established visually, under a light microscope by gently pressing with tweezers on the seed to test for toughness and resistance to pressure. Viable seeds (filled) are swollen throughout with no concave sides, whereas non-viable seeds (unfilled) are thin and flat and contain no embryo (Benefield et al., 2001; Hierro et al., 2009). Preliminary tests involving seed dissection and germination of seeds collected in the wild allowed us to determine seed viability based on these attributes. We counted total ovule numbers and seed set of 1,944 pollinated maternal capitula from 314 plants, including, 225 spontaneous self-pollination, 313 manual self-pollination, 265 within population, 263 between populations within region and 878 between region crosses.

2.4 | Data analysis

All analysis were conducted in R v3.5.2. Data on proportion of viable seeds versus total number of ovules per capitula (seed:ovule ratio) were analysed with generalized linear mixed models (GzLMMs) as implemented in the *glmer* function of the LME4 package in R (Bates et al., 2015), assuming binomial distribution. To test for differences in seed ovule ratios between pairs of regions, we used treatment as a fixed factor with individual mother nested in the maternal population and individual father nested in the paternal population as random factors. Differences in seed:ovule ratio between native versus non-native ranges were assessed by GzLMMs with range as a fixed factor and region as a random factor. If the model indicated significant differences among ranges, we applied Tukey HSD post-hoc tests with *p* values < 0.05 to infer which regions differed, using the MULTCOMP package (Hothorn et al., 2008). Effect size was estimated by calculating Cohen's d_s statistics which accounts for unequal group size between the two groups compared (Cohen, 1988).

Data on manual selfing rates were grouped per individual plant and included the average seeds produced after selfing and after outcrossing (Table S2). We then calculated the self-compatibility index (SCI; Lloyd & Schoen, 1992) for every region and each individual plant after the manual selfing treatment based on the formula below:

$$\text{SCI} = \text{seed-set after selfing}/\text{seed-set after outcrossing}, \quad (1)$$

SCI ranges from 0 to 1, with 0.75 as the threshold for a plant to be considered self-compatible (Lloyd & Schoen, 1992).

We also calculated an index of fecundity, adapted after Ramsey et al. (2003), by dividing the mean seed ovule ratio (S:O) value of the between-region cross to the mean S:O value of the within-population within-region cross (control) based on the formula below:

$$\text{FI} = 1 - (S_{between}/O_{between})/(S_{within}/O_{within}), \quad (2)$$

where $S_{between}$ and $O_{between}$ indicate the number of viable seeds and total ovules per capitulum for the between-region crosses, and S_{within}

and O_{within} indicate the number of viable seeds and total ovules for the within-region crosses, respectively. The index ranges from 1 (complete reproductive isolation), through 0 at random mating, and to $-\infty$ when gene flow is facilitated.
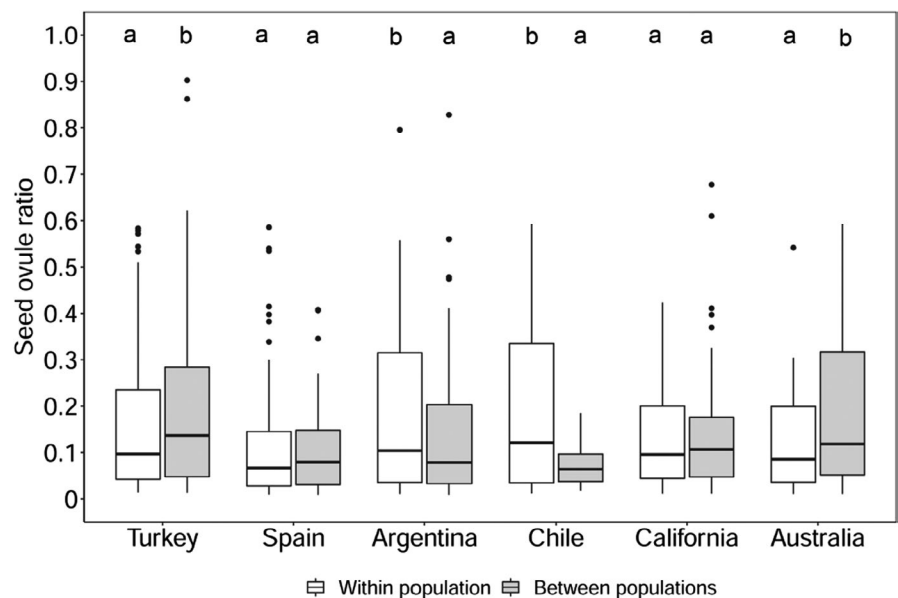
Finally, we tested for isolation by distance using a Mantel test (Pearson correlation, 1,000,000 Monte Carlo simulations, $p < 0.05$) in XLSTAT (XLSTAT, 2019). The geographical distance matrix was generated with the 'dist' function in R to calculate Euclidean physical distance between regions while the fecundity matrix included the region pairwise differences in least squares means for seed to ovule ratio. The pairwise genetic distances (F_{ST}) between populations (except Australia, which currently lacks any genetic resources) were retrieved from a previous genetic study of *C. solstitialis* (Eriksen et al., 2014), as the populations we sampled here largely overlap with the geographical area covered in that study (i.e. Turkey: Mediterranean and Central Anatolian region; Spain: Castile and León and Castile-La Mancha; Argentina: La Pampa, Chile: Santiago; and California: San Francisco and Sacramento area).

3 | RESULTS

3.1 | Admixture effects for crosses within region

Plants from Spain and California displayed similar seed:ovule ratios for the two types of crosses within region. In Argentina and Chile, crosses between distinct populations were disadvantageous relative to crosses within population, resulting in 26% and 59% decreases in seed set, respectively. Conversely, crosses between distinct populations in Turkey and Australia showed 22% and 47% increases in fecundity compared to crosses within populations (Figure 2). For detailed statistics, see Table S3 in Supporting Information.

3.2 | Admixture effects for crosses between regions

Globally, native mother plants displayed higher fecundity rates than non-native plants ($\chi^2_{(1)} = 5.97$, $p = 0.01$, $d = 0.167$). The two type of crosses (within region vs. between region) differed significantly for native versus non-native range ($\chi^2_{(1)} = 38.58$; $p < 0.001$). For crosses within region, both native and non-native mother plants demonstrated similar fecundity rates, whereas for crosses between regions, native plants showed a significant increase in fecundity compared to non-native (Figure 3).

Half of the pairwise controlled crosses between different geographic regions (i.e. 15 out of 30) exhibited pronounced change in fecundity relative to crosses within population (control), with seven positive interactions (heterosis: increased fecundity) and eight

FIGURE 3 Seed to ovule ratio: comparison between native and non-native ranges. Box plot showing the median (continuous horizontal line) and geometric error bars with different letters above boxes indicating significant difference between treatments at $p < 0.05$
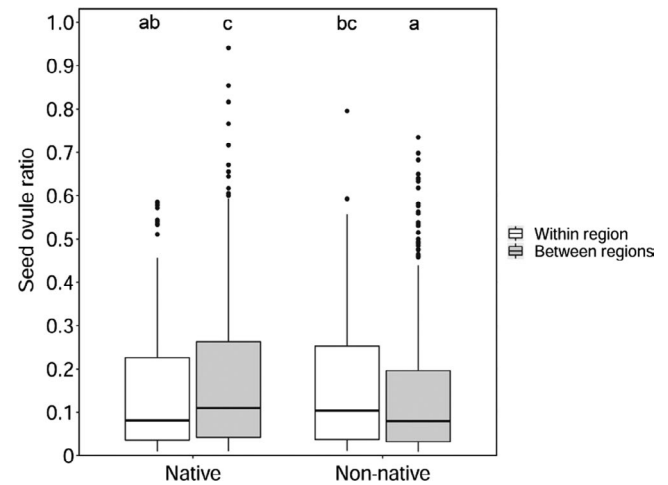
FIGURE 2 Seed to ovule ratio for *Centaurea solstitialis* in treatments within region (comparison between crosses within population vs. crosses between populations). Boxplots indicate median and geometric error bars and different letters above boxes indicate significant differences between treatments within each region at $p < 0.05$. Seed to ovule ratio is given as the percentage of ovules that developed into viable seeds from the total number of ovules available in the capitula treated

	Turkey	Spain	Argentina	Chile	California	Australia
Turkey	—	0.01	−0.26	**0.42**	−0.00	0.03
Spain	−0.16	—	**−0.52**	**−0.48**	**−0.43**	**−0.68**
Argentina	0.07	**0.41**	—	0.10	**0.33**	0.21
Chile	**0.17**	0.00	**0.14**	—	**0.30**	0.25
California	0.11	**0.22**	0.12	**0.48**	—	**−0.18**
Australia	−0.04	0.01	**−0.43**	−0.02	**−0.29**	—

TABLE 1 Pairwise matrix of fecundity indices for *Centaurea solstitialis*. The index ranges from 1 (complete reproductive isolation), through 0 at random mating, to −∞ when gene flow is facilitated. Bold numbers indicate statistically significant differences ($p < 0.05$). Columns indicate the region of origin of the paternal genotypes, and rows indicate the origin of the maternal genotypes
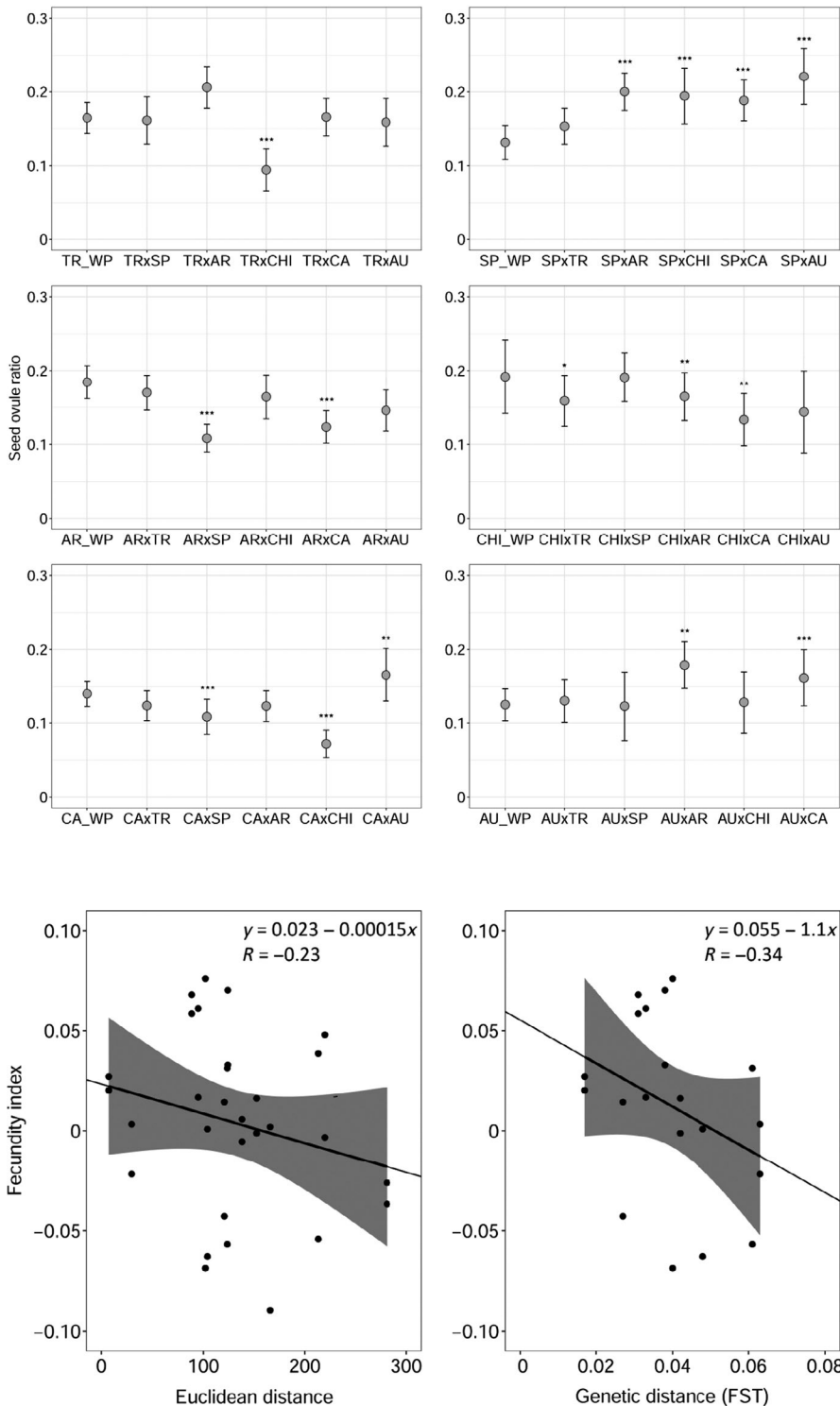
FIGURE 4 Seed to ovule ratio in *Centaurea solstitialis* for treatments between regions, and comparison to within populations within region values (control), for each of the six maternal regions and their pairwise combinations expressed as means and SEs of the means. Geographical regions are abbreviated by two or three (Chile) letter country codes and treatment within population is abbreviated as WP. Significant deviations from control distributions are indicated as: ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$

FIGURE 5 The relationship between fecundity index and geographical distance (Euclidean) and fecundity index and genetic distance (F_{ST}) in *C. solstitialis*. Each point represents one pairwise comparison in the cross experiment. Regression line and regression equation are also given for visualization and shading area indicates 95% confidence intervals

negative interactions (decreased fecundity; Table S4). Native-range individuals from Spain demonstrated the highest number of positive fitness interactions with significant increase in fecundity whenever pollen from non-native range was used (Table 1; Figure 4). In contrast, populations from the Americas displayed mostly negative fitness interactions, indicative of reproductive incompatibilities when treated with pollen from the native range as well as with pollen from other non-native regions (Table 1; Figure 4). Australia was the only non-native region that exhibited positive fitness interactions when crossed with pollen from the Americas. The indices of fecundity for crosses between regions ranged between 0.14 and 0.48 (i.e. reproductive incompatibility) to −0.18 and −0.68 (increased fecundity; Table 1).

3.3 | Isolation by distance

No significant correlation was found between geographical distance and fecundity indices ($r = -0.13$, $p = 0.31$) nor between genetic distance and fecundity indices ($r = -0.38$, $p = 0.13$), based on Mantel test (Figure 5; Table S5).

4 | DISCUSSION

Experimental crosses within and between native and non-native regions of the invasive *C. solstitialis* resulted in highly variable fitness outcomes, indicating distinct patterns of gene flow among geographical ranges. Neo-allopatric regions showed a prevalence of selection against distant pollen or reinforcement; in contrast, some native regions demonstrated an increase in fecundity, a pattern that was not correlated with spatial or genetic distance between regions. Also, we found that *C. solstitialis* had not experienced shifts in its reproductive system from outcrossing to self-fertilization, when moving from the native to the non-native range.

Admixture of different source populations during species introductions is hypothesized to facilitate the adaptive potential of introduced populations by creating novel genetic combinations that can boost fitness in the newly colonized habitats and play an important role in the invasion process. Still, there is conflicting evidence about the importance of admixture to invasion and little research about its evolutionary implications (Rius & Darling, 2014; Vellend et al., 2007). Admixture benefits are expected to be maximal during recent invasion and decline in magnitude over time, as genetic dissimilarity between populations increases (Barker et al., 2019). *Centaurea solstitialis* has a well-documented colonization history in its non-native range (Eriksen et al., 2014). The species expansion from out of the native area has been linked to the alfalfa farming. An increase in demand for this crop during the first two decades of the twentieth century lead to extensive international trade and massive imports of cheap alfalfa seeds into the Americas from various sources in Eurasia, which was contaminated with different weed seeds, including those of *C. solstitialis* (Gerlach Jr., 1997). Recent genetic analysis based on

genome-wide SNPs markers provide support that *C. solstitialis* introductions to Chile and California originated from a single source gene pool of Spanish origin, and involved weak genetic bottlenecks (Barker et al., 2017). In contrast, populations in Argentina appear to be derived from multiple introductions of different source populations in Chile, France and Syria (Hijano & Basigalup, 1995). Likewise, there is an indication that populations in western Europe are derived from an ancient admixture event between populations from eastern Europe and Asia (Barker et al., 2017). Clear genetic differentiation was found among populations in Eurasia but little genomic structure in the introduced range (Barker et al., 2017). Thus, admixture might have been a potential driver of the colonization success in this species.

In contrast to the history of colonization, limited information is available about population connectivity and rates of gene flow in *C. solstitialis* populations in each of its native and introduced range. For example, genetic studies found low interpopulation divergence for *C. solstitialis* in the western US (Sun, 1997) and no population substructure in South America (Eriksen et al., 2014). Our crosses within region revealed evidence of genetic drift and inbreeding in Turkey and Australia, where seed set benefited from crosses between far populations, and local adaptation or selection against distant pollen in Argentina and Chile, where seed set decreased in crosses between geographically distinct populations. Conversely, we found no difference in reproductive success for populations in Spain and California, suggesting high rates of gene flow in these regions. In the native range, but also in some parts of the introduced range such as Chile and Australia, *C. solstitialis* occurs in small patches, at low densities, with populations often many km apart from each other (Uygur et al., 2004), which is in contrast to invasive populations in Argentina and California that form very large and dense stands (Andonian et al., 2011). Seed and pollen dispersal for this species is usually limited to the proximity of the source population, although birds, animals and humans can be implicated in long distance gene flow and thus promote admixture between populations (Miguel et al., 2017; Roché, 1992). A previous study testing for reproductive isolation in this species found a 25% decrease in seed set for plants pollinated by a different Californian population than by plants from the same population (Montesinos et al., 2012). The dissimilarity between the two studies might be attributed to a methodological difference as here we analysed the seed to ovule ratio, whereas in the original experiment (Montesinos et al., 2012), only the number of viable seeds was measured.

Compared to within region crosses, admixture benefits were higher for between region crosses, but only for native populations. We found partial support for our first prediction. Populations in the native range developed mostly neutral or positive fitness interactions when treated with pollen from other regions, with just one negative interaction—the cross between Turkish maternal genotypes and Chilean paternal genotypes. The large increase in reproductive success when Spanish populations served as the maternal parent in crosses with all other non-native regions might be explained by the fact that populations in this region were the primary contributors of seeds that colonized the Americas, potentially serving as a 'bridgehead' (Barker et al., 2017, 2019). The invasive 'bridgehead

effect' refers to a particular population that serves as the proximate origin of successful introductions into several other areas (Lombaert et al., 2010). A previous study examining admixture effects in the native range of *C. solstitialis* by Barker et al. (2019) also found very strong positive fitness interactions in F_1 progenies derived from maternal genotypes of Spanish plants, as was the case in our study. *Centaurea solstitialis* populations in Western Europe are the result of a past admixture event between Asian and eastern European populations (Barker et al., 2017), but for some reason, experimental crosses in this region seem to provide additional fitness benefits.

Experimental admixture in the non-native ranges resulted in mostly negative fitness interactions, with the exception of Australia, lending support to our second prediction. Populations in the Americas were all (except Chile) equally compatible with pollen from Turkey, but showed reduced ability to produce viable seeds while receiving pollen from Spain, suggesting reinforcement. *Centaurea solstitialis* populations in the Americas have a relatively recent history, being introduced ca 400 years ago in Chile and <200 years ago in Argentina and California (Gerlach Jr., 1997; Hijano & Basigalup, 1995; Pitcairn et al., 2006), primarily from seeds of Spanish origin (Barker et al., 2017), although secondary introductions were also documented (Gerlach Jr., 1997; Hijano & Basigalup, 1995). There is little genetic divergence among these populations, relative to the native source (Spain; Barker et al., 2017; Eriksen et al., 2014). Nonetheless, a compelling body of literature indicates that populations in the Americas have evolved adaptively, which has led to the accumulation of different ecological fitness benefits compared to native genotypes (e.g. Barker et al., 2017; Eriksen et al., 2012; Filipe & Montesinos, 2016; García et al., 2013; Graebner et al., 2012; Montesinos & Callaway, 2017, 2018; Montesinos et al., 2019) and to the emergence of incipient reproductive incompatibilities (Montesinos et al., 2012). Populations in Chile and Argentina showed reduced compatibility to pollen from Californian plants, with Chile exhibiting reduced fecundity also to pollen from Argentina. Additionally, a bidirectional decrease in seed set was observed between Chile and California. Previous genetic studies have found that *C. solstitialis* populations in California are distinguished from those in South America (Barker et al., 2017; Eriksen et al., 2014), suggesting that genetic distance could contribute to the reduced fecundity observed between these regions. However, this do not hold true for populations in South America which appear to be genetically homogeneous (Barker et al., 2017; Eriksen et al., 2014). In contrast to the rest of the non-native regions, Australia was the only region unaffected by pollen source. The population structure and colonization history of *C. solstitialis* in Australia, where the species is believed to have been introduced around 1892 (Kloot, 1983), have not yet been resolved with molecular data. Our findings suggest that Australia might have been colonized from seeds of Spanish origin or elsewhere in the invaded range. Our results also suggest that multiple introductions in the Americas have already lead to admixed populations and experimental crosses in this region do not further increase fitness. Interestingly, contact between Australian and American populations seems to further boost fitness. These findings are consistent with observations in other invasive plants such as *Lythrum salicaria*

where crosses between some invasive range populations lead to heterosis and an increase in total plant biomass (Shi et al., 2018).

Our study found an asymmetrical response to inter-regional gene flow suggesting the presence of multiple evolutionary mechanisms across *C. solstitialis* range. Contrary to our third prediction, fecundity rates were not related to spatial distance nor genetic distance between populations, ruling out isolation by distance as a dominant factor to the reproductive patterns observed. Environmental variation is also unlikely to be responsible for the observed pattern. *Centaurea solstitialis* appears to have retained a similar climatic niche across native and non-native ranges, although invading populations in California seem to occupy the warmest and driest habitats available, which in the native range are less common (Dlugosch, Cang, et al., 2015). Climatic factors such as seasonal variation in temperature and annual precipitation were found to impact several plant traits such as growth, resistance to drought and effective population size, but were not correlated to reproductive success in this species (Braasch et al., 2019; Dlugosch, Cang, et al., 2015). Based on those findings, we can exclude isolation by environment among *C. solstitialis* populations and infer that external factors do not seem to constitute a major barrier to gene flow in this species. Furthermore, we suggest the presence of intrinsic barriers to gene flow, with admixture potentially disrupting locally co-adapted allele combinations via negative epistasis (interaction of alleles at different loci; Barker et al., 2019). Another study testing the effects of admixture on the first generation of hybrids between different source populations of *C. solstitialis* from the native range concluded that both the identity of the maternal parent and the genetic diversity present within the maternal population were important for the performance of the F_1 hybrids (Barker et al., 2019). They also hypothesized that some negative fitness effects may be due to interactions between the nuclear and uniparentally inherited cytoplasmic genomes (i.e. cyto-nuclear incompatibilities; Barker et al., 2019). Our study is partially consistent with that hypothesis, with six pairs of reciprocal crosses generating asymmetrical seed production with reduced reproductive success when the maternal parent came from the Americas. Other factors that could influence seed output such as negative pollen–pistil interactions or structural modification in floral morphology appear to be absent in this species (D. Montesinos, pers. obs.). A potential confounding factor is that we did not specifically explore for variation in seed production among earlier and later capitula of the same plant. Since *C. solstitialis* is non-clonal, inflorescences are physiologically integrated, and this should ensure access to a constant amount of resources, with ovule number being the ultimate limiting factor in seed production. The species is known to produce a constant number of ovules per inflorescence (Swope, 2014), which was confirmed in our study. Moreover, our plants were not water or resource limited, and received abundant pollen. Another factor that might have influenced our results could be environmental maternal effects, but since we grew our plants under common conditions, maternal effects on fecundity were probably minor. In other common garden studies that tested for maternal effects in *C. solstitialis*, only small differences in seed attributes and germination rates were found between the progeny of wild sampled plants and the progeny of cultivated plants

(Hierro et al., 2009; Widmer et al., 2007). Nevertheless, the mechanisms behind reduced cross fecundity requires further investigation of F_1 seeds germination, seedling survival and fitness. Ultimately, screening for genetic incompatibilities in subsequent generations of hybrids resulting from these crosses could reveal if the cross incompatibilities are active at the pre-zygotic or post-zygotic stages and whether they are caused by single or multilocus incompatibilities.

In conclusion, our study revealed a biogeographical mosaic of variable fitness outcomes among experimental crosses between geographically disjunct regions of *C. solstitialis*, with indication of heterosis in some native regions and incipient reproductive incompatibilities in some non-native regions. These results expand current evidence of rapid evolution in this invasive species after relatively short times since introduction into new ranges. Our results also support the idea that, at this stage of invasion admixture in some *C. solstitialis* non-native regions (Americas) might have detrimental effects on local populations, but that in others (Australia) as well as in the native Spain, admixture could result in increased fecundity and represent a risk of boosting invasion.

AUTHORS' CONTRIBUTIONS
R.E.I. and D.M. designed the study; R.E.I., D.M. and S.B. performed the experiments; R.E.I. and D.M. analysed the data; D.M., J.L.H., G.S., L.A.C., O.E., C.J.L., K.F. and R.M.C. collected plant seeds in the field; R.E.I. and D.M. wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

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