Increments in weed seed size track global range expansion and contribute to colonization in a non-native region

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Abstract Assessing global variation in phenotypic traits and linking that variation to colonization and range expansion is notably rare in invasion biology. Here, we studied variation in seed size in *Centaurea solstitialis*, a weed with worldwide distribution. Additionally, we explored how seed size variation affects seedling survival of *C. solstitialis* under favorable precipitation conditions in Anatolia, an ancestral region, and unfavorable precipitation conditions in Argentina, a non-native region. To that end, we conducted seed collections following dispersal pathways of *C. solstitialis* in ancestral, expanded, and non-native ranges. Locally, collections followed elevation gradients. Also, we performed a greenhouse experiment with *C. solstitialis* populations varying in seed size and water additions simulating precipitation patterns in Anatolia and Argentina. Seeds from ancestral populations at low elevation were smaller than those from the rest of study populations. Also, seed size in populations at high elevation in the expanded range, the main source of non-native populations, was similar to that in all, but one non-native population, where seeds exhibited further increase. Increments in seed size thus track range

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expansion in *C. solstitialis*. Locally, seed size increased with elevation in all three ranges, suggesting convergent responses to that gradient. Seedlings from larger seeds displayed greater survival than those from smaller seeds only under Argentinean conditions. Consequently, populations with large seeds may have been instrumental for colonizing that non-native region. Our findings suggest that ancient and recent dispersal of large-seeded populations contribute to explain the reported global pattern of seed size divergence and worldwide distribution of *C. solstitialis*.

Keywords *Centaurea solstitialis* L. · Dispersal pathways · Introduction sources · Parallel clines · Seedling survival · Trait variation

Introduction

In a biogeographical sense, dispersal allows species to reach sites where they are not present (Lomolino et al. 2017). Dispersal thus gives species the chance to colonize new areas and expand geographical ranges. Because those chances are low, dispersal is considered as a rare biogeographical process, albeit with enormous consequences for species distribution (Lomolino et al. 2017). Humans have dramatically altered the exceptional nature of dispersal. In fact, the assistance that humans provide to other species to reach sites beyond their dispersal abilities (i.e., species/biological introductions/invasions) is a major component of current global change (Vitousek et al. 1997). An impressive and rising number of studies have been devoted to understanding the mechanisms driving colonization and range expansion upon human-

mediated dispersal (Catford et al. 2009; MacDougall et al. 2018; Pearson et al. 2018). Research on phenotypic trait variation occupies a central place within that work (Bossdorf et al. 2005; Hodgins et al. 2018; van Kleunen et al. 2018), mirroring its centrality in studying the processes influencing the natural expansion of distributional ranges (Kawecki 2008; Blanquart et al. 2013).

Seeds are fundamental units of dispersal (Howe and Smallwood 1982), and increments in seed size could facilitate colonization and expansion in non-native ranges. For example, seeds have been shown to be larger in non-native than native populations (Buckley et al. 2003; Widmer et al. 2007; Graebner et al. 2012; Hierro et al. 2013), and larger seeds have been found to produce larger seedlings (Widmer et al. 2007; Graebner et al. 2012) with increased competitive resistance (Graebner et al. 2012) and survival (Hierro et al. 2013) as compared to smaller seeds. Except for the pioneering work of Buckley et al. (2003), there is, however, no information about how seed size varies across distributional ranges. Moreover, global assessments of variation in phenotypic traits and links of that variation to range expansion are in general notably rare in invasion biology (but see Hierro et al. 2009; Barker et al. 2017). Providing that information may shed light into mechanisms leading to establishing broad geographical distributions.

Early theoretical models predict that increased seed size improves plant fitness under unfavorable conditions (e.g., shaded, dry environments), whereas fitness is independent of seed size under favorable ones (e.g., open, moist environments; Venable and Brown 1988). According to this proposition, large seeds promote initial seedling reserves and size, improving survival relative to small seeds in unfavorable environments, but large seeds do not provide a survival advantage to seedlings as compared to small seeds when environmental conditions are favorable. That is, most seedlings, regardless of the size of the seed they come from, are expected to survive in favorable environments (Westoby et al. 1992). The empirical exploration of this idea has provided mixed results (studies cited in Venable and Brown 1988; Mazer 1989; Leishman and Westoby 1994; Leishman et al. 2000; Caddick and Linder 2002; Moles and Westoby 2004, 2006; Peco et al. 2009; Suárez-Vidal et al. 2017). In the context of invasions, we are aware of only one study assessing demographic consequences

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of seed size in favorable versus unfavorable environments (Hierro et al. 2013). In reciprocal common gardens experiments, Hierro et al. (2013) showed that *Centaurea solstitialis* seedlings derived from the large seeds of non-native Argentinean populations established at greater proportions than seedlings from the small seeds of native Anatolian populations only in Central Argentina, where the precipitation regime poorly matches the natural emergence of the species (unfavorable environment), whereas seedlings from large and small seeds established at similar proportions in Southwestern Anatolia, where the precipitation matches the emergence of the species (favorable environment). These findings provide support to theoretical expectations and raise the possibility that populations with large seeds were instrumental for the colonization of *C. solstitialis* in Central Argentina. Because Southwestern Anatolia and Central Argentina differ in a number of conditions in addition to precipitation patterns, the role of the latter in the establishment of *C. solstitialis* seedlings remains conjectural.

Here, we studied global variation in seed size in *C. solstitialis* and the importance of that variation for the colonization of the species under precipitation patterns in Southwestern Anatolia and Central Argentina. We explored trait variation by conducting seed collections across the distributional range of that species, tracking its dispersal routes reconstructed from genetic studies (Eriksen et al. 2014; Barker et al. 2017; Fig. 1a). At a regional scale, we conducted collections along elevation gradients because seed size has been reported to vary with that environmental gradient (e.g., Pluess et al. 2005; Guo et al. 2010). That approach allowed us to investigate the development of parallel elevation clines in seed size throughout the distribution of *C. solstitialis* (e.g., Alexander et al. 2009). In addition, we assessed environmental and genetic effects of elevation on seed size by growing individuals from ancestral populations located at low and high elevation under common conditions in a greenhouse. Using ancestral populations, we also assessed the heritability of seed size in *C. solstitialis* by means of parent–offspring analysis (Conner and Hartl 2004) to gain insight into the evolutionary potential of that trait. Finally, to evaluate the significance of seed size in the colonization of *C. solstitialis* under different precipitation conditions, we performed a greenhouse experiment with *C. solstitialis* populations varying in seed size and water additions simulating precipitation patterns in Southwestern Anatolia and Central Argentina (Fig. 2). We predicted that seedlings emerging from large seeds will exhibit greater survival than those emerging from small seeds only under the treatment simulating precipitation in Central Argentina.

Materials and methods

Study system

Centaurea solstitialis L. (yellow starthistle; Asteraceae) is an annual weed (technically, a ruderal) that originated in the Caucasus and Anatolia regions (ancestral range; Maddox et al. 1985; Eriksen et al. 2014). From there, the species expanded its range, presumably by diffusion, to Europe and other regions in Asia (expanded range; Eriksen et al. 2014; Barker et al. 2017). Both ancestral and expanded ranges are commonly considered as the native range of *C. solstitialis* (Hierro et al. 2009; Montesinos et al. 2012). In the second half of the nineteen century, *C. solstitialis* was widely dispersed around the globe probably as a contaminant of agricultural seeds (Maddox et al. 1985; Gerlach 1997). As a result of this recent human-assisted range expansion, the species is now present in North and South America, Africa, and Australia (non-native range). Dispersal pathways inferred from historical records of seeds imported for agriculture (i.e., alfalfa seeds) indicate that the main region providing seeds to South America (Argentina and Chile) was Western Europe (Spain, France, and Italy; Gerlach 1997; Hijano and Basigalup 1995). This dispersal pathway has recently been confirmed by molecular evidence (Eriksen et al. 2014; Barker et al. 2017; Fig. 1a). Importation records additionally show that Argentina received agricultural seeds from Syria (Hijano and Basigalup 1995), which is also in agreement with genetic information suggesting that contributions from the ancestral range to South America were important (Eriksen et al. 2014). Populations in North America (California), in turn, seem to largely derive from those in South America (Eriksen et al. 2014; Barker et al. 2017). Finally, there is strong evidence indicating that populations in Western Europe originated from ancient admixture

Fig. 1 a Global distribution and dispersal routes of *Centaurea solstitialis*. Our study sampled regions within the ancestral (dark gray oval), expanded (gray oval), and non-native (light gray ovals) range, except for South Africa. Arrows indicate dispersal pathways reconstructed from genetic studies. The weight of those arrows shows the importance of contributions from expanded and ancestral ranges to South America. Sources of Australian populations are uncertain. **b** Pappus and non-pappus seed size across study populations of *C. solstitialis*. Circles are means ± SEs based on the number of populations indicated in Table 2. Different letters indicate significant differences between regions for both seed morphs combined at $p < 0.05$ after pair-wise comparisons adjusted with Bonferroni. Similarly, the asterisk shows significant differences between pappus and non-pappus seeds. H, high elevation populations; L, low elevation populations
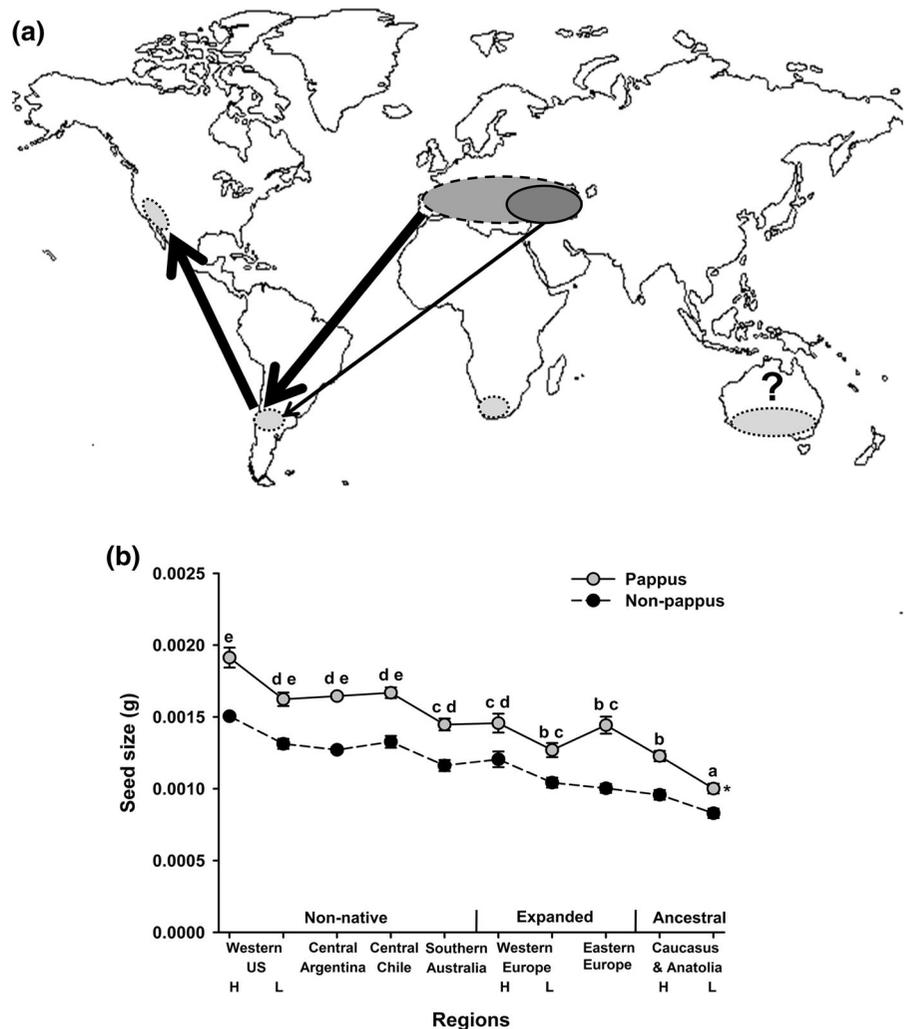
events between populations in Eastern Europe and Asia (Barker et al. 2017).

The worldwide distribution of *C. solstitialis* encompasses environments with varying precipitation regimes. Some of the regions where *C. solstitialis* grows are characterized by a Mediterranean-type climate with wet winters and dry summers, whereas other regions have a precipitation regime in which most of the precipitation falls during the summer with substantially drier winters (Hierro et al. 2009). Among the latter regions, Central Argentina offers the most extreme example documented up to date (Hierro et al. 2009). In all regions, *C. solstitialis* seedlings emerge in the fall (Sheley and Larson 1994; Hierro et al. 2006); thus, winter conditions may affect the survival of the species (Hierro et al. 2009, 2013). Accordingly, it has

been argued that *C. solstitialis* germination and emergence are well synchronized to a Mediterranean-type climate (Joley et al. 2003; Fig. 2). *Centaurea solstitialis* produces two types of seeds (technically, achenes). Peripheral, outer seeds are smaller, darker, and do not have a pappus; whereas central, inner seeds are larger, cream-colored, and bear a permanent pappus (Maddox et al. 1985; Benefield et al. 2001; Miguel et al. 2017).

Global patterns of seed size variation

Seed collections were conducted to explore global patterns of seed size variation in *C. solstitialis*. Collections followed the purported dispersal pathways of *C. solstitialis*, and covered almost the entire
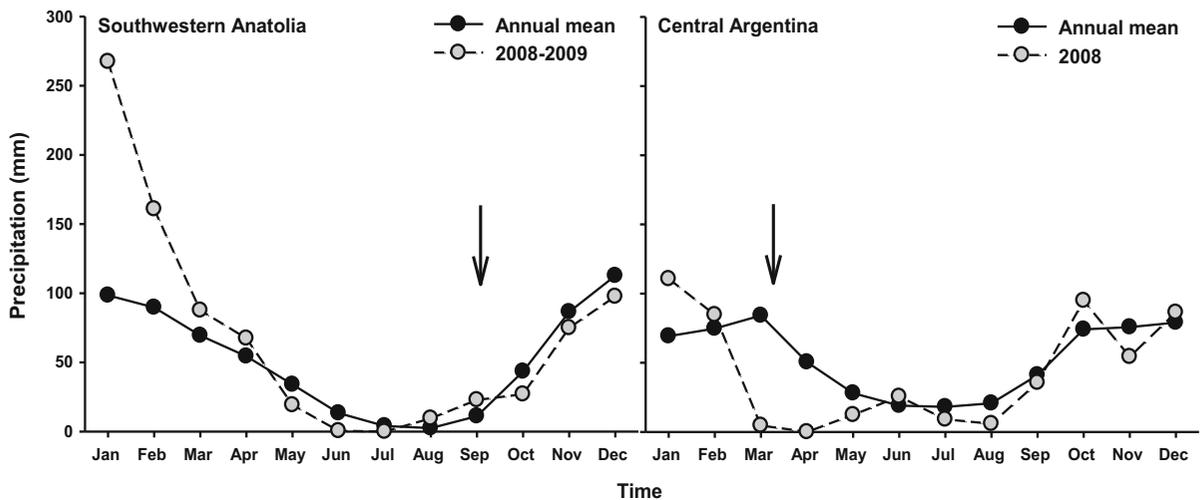
Fig. 2 Historic mean annual precipitation and precipitation during the reciprocal common garden reported in Hierro et al. (2013) for Aydin, Southwestern Anatolia (1971–2010), and Santa Rosa, Central Argentina (1911–2010). Arrows indicate the emergence time, autumn, of *Centurea solstitialis* in both regions. The emergence of *C. solstitialis* is well synchronized to a Mediterranean climate, such as that in Southwestern Anatolia.

The emergence of the species in Central Argentina, however, occurs at the verge of the beginning of the dry winters characterizing the climate in that region. Winter months were particularly dry during the year in which the common garden was conducted in Central Argentina; in contrast, winter was wetter than the mean that year in Southwestern Anatolia. These observations provide the basis for the hypothesis addressed here

distribution of the species, including populations in the ancestral range (Caucasus and Anatolia), expanded range (Eastern and Western Europe), and all non-native regions, except for South Africa (i.e., Central Argentina, Central Chile, Southwestern US –California-, and Southeastern Australia). Within each sampling region, collections were conducted along an elevation gradient. Seeds were collected between 2001 and 2014, and totaled 83 populations in the ancestral range (18 in the Caucasus—5 in Armenia and 13 in Georgia—and 65 in Anatolia—Turkey), 11 in Eastern Europe (8 in Romania and 3 in Hungary), 14 in Western Europe (13 in Spain and 1 in France), 73 in Argentina, 12 in Chile, 14 in California, and 8 in Australia (Online Resource 1). In all cases, collections were performed during seed dispersal (July–September and December-January in the Northern and Southern hemisphere, respectively) from 2 to 54 individuals (one population in Australia and Spain, respectively) per population, which were separated by a minimum distance that varied from 2.5 km (Chile) to 168 km (Spain) during each sampling season. Seeds of each individual plant were kept separated in paper bags, except for collections in France, where seeds were pooled within the single population harvested

there. In all cases, seeds were weighed within 1 year of collection.

Environmental effects on seed size variation and seed size heritability

To assess environmental effects on seed size variation along the elevation gradient, a common garden experiment was conducted in a greenhouse. In addition, this experiment served to evaluate the heritability of the trait in *C. solstitialis*. The greenhouse was located at the Agronomy Campus of the Adnan Menderes University (N 37° 45′ 43″, E 27° 42′ 15.76″), 15 km from Aydin, Turkey. Because the greenhouse was not climate controlled, the timing of the experiment matched the natural emergence time of the species. That is, the experiment was started in late October 2014 and ran for 242 days until late June 2015. Temperature inside the greenhouse (23.279 °C ± 8.673, mean ± SD) was nearly identical to that outside the greenhouse (23.400 °C ± 8.522) throughout the experiment (Online Resource 2). A subsample of the *C. solstitialis* populations surveyed in Southwestern Anatolia in 2014 (n = 14) was used for these explorations. Populations were selected as to encompass the entire

elevation range in Southwestern Anatolia (Online Resource 1). Within populations, four individuals were randomly chosen, and pappus seeds from a single individual were added to a single pot. The total number of pots was 56 (14 populations * 4 individuals). Final sample size was 48 pots due to plant mortality and late or no flower production. These causes similarly reduced the final number of populations in the progeny to 13. Plants were grown in 2.5 L plastic pots filled with a mixture of natural grassland soil collected from the Agronomy Campus and fine commercial sand in a 3:1 proportion (Miguel et al. 2017), resulting in a texture of 74% sand, 22% silt, and 4% clay. Soil was passed through a 710 μm mesh sieve to reduce the presence of unwanted seeds. Upon flowering, and throughout pollen production, one individual was cross-pollinated with another individual within populations by gently rubbing flowers against each other, as *C. solstitialis* flowers are predominantly self-incompatible (Sun and Ritland 1998). Cross-pollination was ended when flowers changed color from bright to pale yellow, upon which inflorescences were bagged using 0.06 m × 0.09 m cotton bags to collect the seeds when matured. Individuals that were not crossed between each other were kept 2 m apart during the flowering time to reduce chances of accidental pollination. To avoid potential maternal effects when assessing trait heritability, only the seed size of the father was related to that of the progeny (Conner and Hartl 2004). Seed size of the progeny was the mean of five randomly chosen pappus seeds per individual. Plants were watered as needed, and pots were regularly moved to avoid positional effects, throughout the experiment.

Seed size and seedling survival

Finally, to explore how seed size affects seedling survival in *C. solstitialis* under precipitation conditions in Southwestern Anatolia and Central Argentina, another experiment was conducted in the same greenhouse of and simultaneously with the common garden described above. As in Hierro et al. (2013), the experiment ran for 182 d, from late October 2014 to late April 2015. Because populations from Southwestern Anatolia and Central Argentina are known to differ in seed size (Hierro et al. 2013), populations from both regions were included to encompass a broad range in seed size. To employ fresh seeds from both Anatolian

and Argentinean populations, a subsample of the *C. solstitialis* populations surveyed in Southwestern Anatolia and Central Argentina in 2014 were used in this new experiment (Online Resource 1). Populations sampled in Anatolia reached a maximum elevation of 1393 m, and populations from both low ($\leq 1393/2 = 696$ m; n = 7) and high (> 696 m; n = 7) locations were included in the study. Populations in Central Argentina reached only 365 m, and no inclusion based on elevation was considered (n = 5). Because pappus seeds are more abundant than non-pappus seeds in *C. solstitialis* (Benefield et al. 2001; Miguel et al. 2017), seven pappus and two non-pappus seeds of known size were equidistantly planted in each pot. As before, four individuals were randomly selected from each population, and seeds from a single individual were added to a single pot. Plants were grown using the same pot size and substrate as in the previously described experiment. To assess the importance of growing conditions in the survival of seedlings emerging from seeds with different sizes in *C. solstitialis*, pots received one of two water treatments; one simulating the precipitation pattern in Aydin, Turkey (favorable conditions), and the other that in Santa Rosa, Argentina (unfavorable conditions), with mean annual rainfall in October and April as initial conditions, respectively. Mean annual precipitation for each month was obtained from weather stations located in Aydin (1971–2011) and Santa Rosa (1911–2013). Five pots in each water treatment were not seeded, and soil moisture was periodically measured at 12 cm depth in those pots with a TDR100 sensor. Non-seeded pots also served to assess the efficacy of the soil-sieving procedure. The total number of pots in this experiment was 162 [(19 populations * 4 individual plants * 2 water treatments) + 10 pots with no seeds]. Seedling emergence was recorded throughout the experiment to estimate emergence rate with the Timson's index, as Σn, where n is the cumulative emergence proportion at each sampling date (Timson 1965; Baskin and Baskin 2014). Seedling survival was measured as the proportion of the number of seedlings alive at the end of the experiment (6 months, as in Hierro et al. 2013) relative to the number of seeds added to pots at the beginning of the experiment.

Statistical analyses

Data from global seed collections were analyzed with simple linear regressions (SLRs) and linear mixed models (LMMs; Bolker et al. 2008). Regressions assessed the relationship between variations in elevation and seed size within regions (e.g., Guo et al. 2010). The size of pappus seeds for populations in Western Europe was transformed to natural logarithm to comply with the homocedasticity assumption of SLR analysis. Also, data identified as influential according to Cook's distance (Cook 1977) were removed (two populations in Western Europe for both pappus and non-pappus seeds and one in Southern Australia for non-pappus seeds), and analyses were re-run without them. Mixed models compared seed size between populations in ancestral, expanded (Eastern and Western Europe), and non-native regions. These models considered region, seed type, and their inter-action as fixed effects, and population, nested within region, as a random effect. Regions where seed size was found to vary with elevation were grouped into low and high elevation. For grouping, populations located at or below half the maximum elevation reached by populations within the region were considered as belonging to low elevation; populations located above that criteria were considered as high elevation. Low and high elevation groups were introduced into LMMs as separated regions. Significance values of pair-wise comparisons were adjusted with Bonferroni.

Data from greenhouse experiments were also analyzed with SLRs, including evaluations of the effect of variation in elevation on variation in seed size of the progeny, variation in parental seed size (i.e., seed size of the father) on that in progeny seed size (Conner and Hartl 2004), variation in seed size on variation in seedling survival (Cottingham et al. 2005), and variation in emergence rate on that in seedling survival. Survival is expressed as proportion, and it was transformed to the arcsine function for analyses because proportional data are best analyzed using conventional models (i.e., models with normal errors and constant variance) following that transformation (Crawley 2005). Statistical analyses were conducted with SigmaPlot 11.0 (SLRs) and IBM SPSS Statistics 25 (LMMs).

Results

Global patterns of seed size variation

The size of pappus and non-pappus seeds in *C. solstitialis* populations increased with elevation in three regions, Anatolia and the Caucasus, Western Europe, and Western US (Table 1; Fig. 3). Populations in Eastern Europe, Central Chile, Central Argentina, and Southern Australia showed, instead, no relationship between elevation and seed size. Lack of relationship between those variables in Eastern Europe and Argentina were expected, as variation in elevation is limited in those regions (18–357 m and 53–365 m, respectively; Online Resource 1).

Variation in the LMM explained by fixed effects (Online Resource 3) was several orders of magnitude larger than that explained by the random effect (estimate $\pm$ SE = 2.398E$-$8$\pm$1.813E$-$9). Population (region) was, however, significant in the model (Wald Z = 13.224; $p < 0.001$), indicating differences in seed size between *C. solstitialis* populations within study regions. Importantly, seed size in *C. solstitialis* displayed large differences across regions throughout the global distribution of the species ($F_{\text{Region } 9, 392.404} = 48.620, p < 0.001; F_{\text{Seed type } 1, 17,370.606} = 1861.650, p < 0.001$; $F_{\text{Region*Seed type } 9, 17,361.333} = 33.093, p < 0.001$; Fig. 1b; Table 2). Seeds from ancestral populations located at low elevation were smaller than those from the rest of populations sampled in our study ($p < 0.05$). Seeds from ancestral populations at high elevation were, instead, smaller than those from high elevation in Western Europe and all non-native populations ($p < 0.05$). In addition, Western European populations located at high elevation displayed seeds similar in size to seeds from populations in the rest of Europe and populations in all non-native regions ($p > 0.05$), but those in the Western US at high elevation ($p < 0.05$). Seeds from those high elevation populations in the US were larger than seeds from Western European and Australian populations ($p < 0.05$ for both cases). No differences in seed size were detected between populations from the rest of the non-native regions ($p > 0.05$). In total, seed size in *C. solstitialis* exhibited 90% increase from populations growing at low elevation in the ancestral range to non-native populations occurring at high elevation in the Western US ($p < 0.001$). Finally, pappus seeds were

Table 1 Output for simple linear regressions (SLRs) conducted to evaluate seed size variation in *Centaurea solstitialis* along elevation gradients in ancestral, expanded, and non-native ranges

Range	Region	Seed type	n	Slope	t	r^2	F	p
Ancestral	**Caucasus and Anatolia**	**Pappus**	**83**	**3.709E−7**	**6.613**	**0.351**	**43.725**	**< 0.001**
		Non-pappus	**82**	**2.201E−7**	**3.931**	**0.162**	**15.453**	**< 0.001**
Expanded	Eastern Europe	Pappus	11	− 2.849E−7	− 0.400	0.018	0.160	0.698
		Non-pappus	11	3.554E−8	0.093	0.001	0.009	0.928
	Western Europe	**Pappus**	**12**	**0.0004**	**4.197**	**0.638**	**17.614**	**0.002**
		Non-pappus	**12**	**4.945E−7**	**4.341**	**0.653**	**18.842**	**0.002**
Non-native	Central Chile	Pappus	12	4.208E−8	0.410	0.017	0.168	0.691
		Non-pappus	12	1.655E−7	1.660	0.216	2.754	0.128
	Central Argentina	Pappus	73	− 1.851E−7	− 0.883	0.011	0.779	0.380
		Non-pappus	73	− 6.684E−9	− 0.033	1.510E−9	0.001	0.974
	Western US	**Pappus**	**14**	**3.639E−7**	**2.712**	**0.380**	**7.353**	**0.019**
		Non-pappus	**14**	**2.273E−7**	**2.386**	**0.322**	**5.690**	**0.034**
	Southern Australia	Pappus	8	5.874E−8	0.419	0.028	0.176	0.690
		Non-pappus	7	4.714E−7	2.044	0.455	4.177	0.096

Significant relationships are indicated in bold. The number of populations in each region is referred to as n. Two populations identified as influential were removed from analyses of both pappus and non-pappus seeds in Western Europe and one population from analyses of non-pappus seeds in Southern Australia. Analyses of Western European populations for pappus seeds were conducted with data transformed to natural logarithm to comply with the homocedasticity assumption of the SLR analysis

larger than non-pappus seeds across regions ($p < 0.001$).

Environmental effects on seed size variation and seed size heritability

Variation in elevation explained 53% and 42% of the variation in seed size of the progeny for pappus ($r^2 = 0.529$, slope = 4.538E−7, t = 3.512, $F_{1, 12} = 12.332$, $p = 0.005$) and non-pappus seeds ($r^2 = 0.424$, slope = 2.851E−7, t = 2.846, $F_{1, 12} = 8.102$, $p = 0.016$; Fig. 4a), respectively. In addition, variation in paternal seed size explained a modest, but significant, proportion of that in progeny seed size in *C. solstitialis* populations ($r^2 = 0.160$, slope = 0.347, t = 2.963, $F_{1, 47} = 8.778$, $p = 0.005$; Fig. 4b).

Seed size and seedling survival

Seed size variation also explained a large proportion of seedling survival variation and these variables were positively associated under the precipitation pattern of Central Argentina ($r^2 = 0.319$, $F_{1, 18} = 7.978$, $p = 0.012$; Fig. 5), but survival was independent of seed size under the precipitation pattern of Southwestern Anatolia ($r^2 = 0.039$, $F_{1, 18} = 0.680$, $p = 0.421$). Soil moisture in pots throughout the experiment resembled precipitation from October/April to April/October in Aydin, Turkey/Santa Rosa, Argentina, respectively (Online Resource 4). Similar to seed size, seedling survival depended on the rate of seedling emergence only under the treatment that simulated the precipitation of Central Argentina ($r^2 = 0.465$, $F_{1, 18} = 14.801$, $p = 0.001$ and $r^2 = 0.162$, $F_{1, 18} = 3.282$, $p = 0.088$ for Argentina and Anatolia water treatments, respectively; Online Resource 5).

Discussion

Exploring variation in phenotypic traits throughout native and non-native distributions is uncommon in invasion biology (Buckley et al. 2003). Similarly, few studies have related trait variation to colonization and range expansion in species distributed worldwide by humans (Hierro et al. 2009; Barker et al. 2017). Here, we assessed global patterns of seed size variation following dispersal routes in *C. solstitialis* and tested
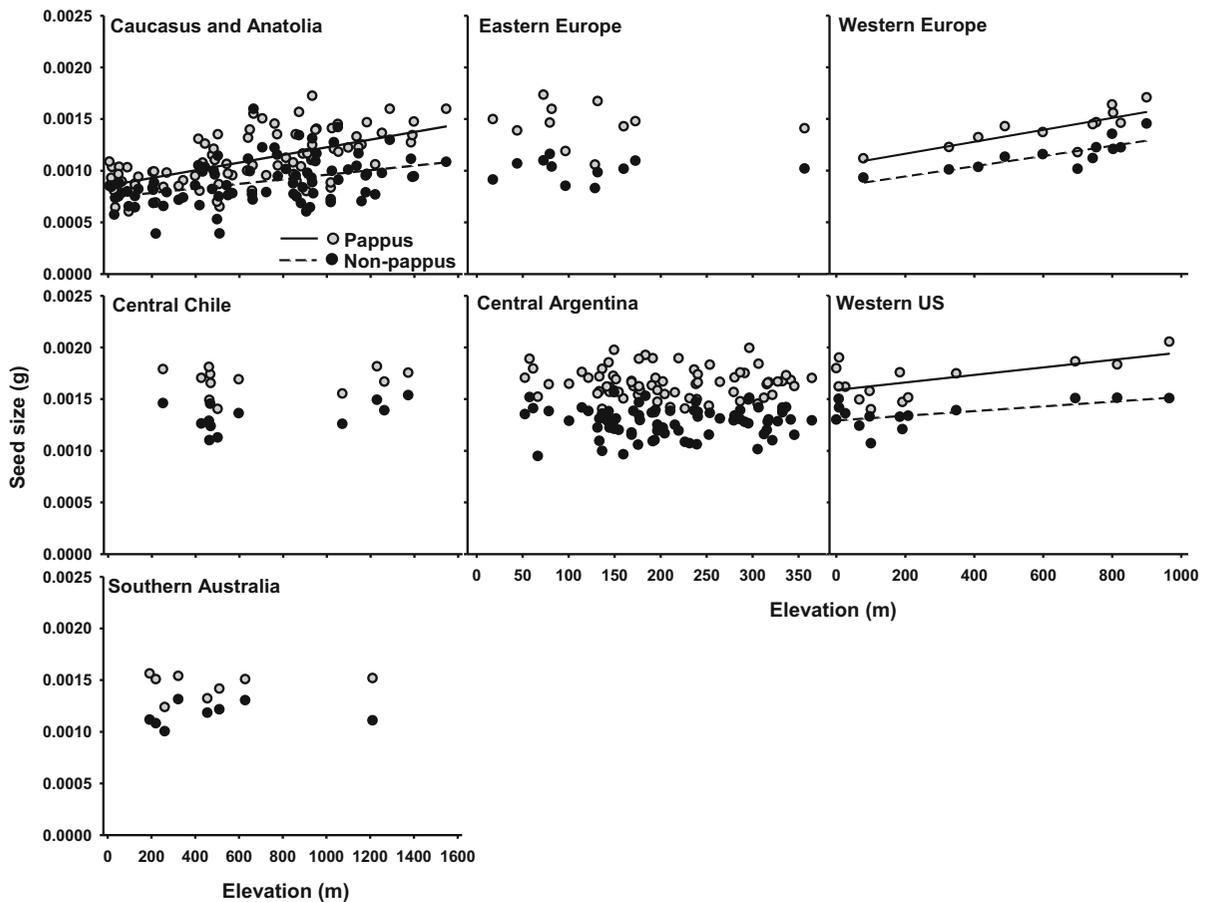
Fig. 3 Seed size variation along elevation gradients in regions within the ancestral (Caucasus and Anatolia), expanded (Eastern and Western Europe), and non-native (Central Chile, Central Argentina, Western United States, and Southern Australia) range of *Centaurea solstitialis*. Seed mass in Western Europe is shown without transformation for comparison purposes

the importance of that variation for the colonization of a non-native region with challenging conditions for the establishment of that species. We found that seed size exhibits large variation across the distributional range of *C. solstitialis*, and that increments in seed size accompany its global expansion. Within the ancestral range, populations located at high elevation displayed larger seeds than those at low elevation. Seed size in those high elevation ancestral populations was similar, in turn, to that in populations in the expanded range, including both Eastern and Western European populations located at low elevation. Finally, populations at high elevation in Western Europe, a major donor of populations in the Americas (Eriksen et al. 2014; Barker et al. 2017), had seeds similar in size to those in populations located in all study non-native regions, but populations at high elevation in the Western US.

Along the dispersal route that goes from ancestral populations at low elevation to non-native populations at high elevation in the Western US, *C. solstitialis* nearly doubled the size of its seeds. In addition, populations with large seeds seem to have contributed to the colonization of *C. solstitialis* in one of the non-native regions, as increments in seed size favored seedling survival under the simulated precipitation conditions of Central Argentina. Our findings suggest that ancient dispersal of large-seeded populations from the ancestral to the expanded range and recent dispersal of populations with further increments in seed size from Western Europe to the non-native range could have influenced both the reported global pattern of seed size divergence and current worldwide distribution of *C. solstitialis*.

Table 2 Descriptive data for seed size (g) of *Centaurea solstitialis* populations across study regions

Range	Region	Elevation	Seed type	n	Mean	SD	Minimum	Maximum
Ancestral	Caucasus and Anatolia	Low	Pappus	45	0.0010	0.0002	0.0006	0.0016
		High		38	0.0012	0.0002	0.0008	0.0017
		Low	Non-pappus	44	0.0008	0.0002	0.0004	0.0016
		High		38	0.0010	0.0002	0.0006	0.0014
Expanded	Eastern Europe		Pappus	11	0.0014	0.0002	0.0011	0.0017
			Non-pappus	11	0.0010	0.0001	0.0008	0.0012
	Western Europe	Low	Pappus	6	0.0013	0.0001	0.0011	0.0014
		High		8	0.0015	0.0002	0.0011	0.0017
	Western Europe	Low	Non-pappus	6	0.0010	0.0001	0.0009	0.0012
		High		8	0.0012	0.0002	0.0009	0.0015
Non-native	Central Chile		Pappus	12	0.0017	0.0001	0.0014	0.0018
			Non-pappus	12	0.0013	0.0001	0.0011	0.0015
	Central Argentina		Pappus	73	0.0016	0.0001	0.0014	0.0020
			Non-pappus	73	0.0013	0.0001	0.0009	0.0016
	Western US	Low	Pappus	11	0.0016	0.0002	0.0014	0.0019
		High		3	0.0019	0.0001	0.0018	0.0021
		Low	Non-pappus	11	0.0013	0.0001	0.0011	0.0015
		High		3	0.0015	0.000002	0.0015	0.0015
	Southern Australia		Pappus	8	0.0015	0.0001	0.0012	0.0016
			Non-pappus	8	0.0012	0.0001	0.0010	0.0013

Regions where seed size varied with elevation were grouped into low or high elevation (see text for grouping criteria). The number of populations sampled is referred to as n

Our global sampling collection additionally revealed that seed size not only increased with elevation within the ancestral range, but also within the expanded and non-native range of *C. solstitialis*. We do not know why that pattern was not observed in Central Chile and Southern Australia, two non-native regions with large differences in elevation, but greater sampling effort is the first step to either confirm or reject the absence of the cline in those regions. Other studies have similarly documented parallel environmental clines in phenotypic traits in native and non-native ranges (latitudinal clines, Huey et al. 2000; Maron et al. 2004, 2007; continental clines, Leger and Rice 2007), including elevation clines in seed size for eight species (Alexander et al. 2009). Our comparison involved not only native and non-native ranges, but also the expanded range. Because the elevation cline in seed size developed in the non-native range and the presence of *C. solstitialis* there is much more recent than in the expanded range, establishing the cline in the latter is not surprising. Parallel clines between distant regions of the world indicate convergent population responses to similar changes in environmental conditions (Thompson et al. 2016), and establishing clines in non-native ranges mirroring those in the native range shows that those responses can be fast (Huey et al. 2000; Maron et al. 2004; Leger and Rice 2007; Alexander et al. 2009).

Increments in seed size with elevation in ancestral populations of *C. solstitialis* were maintained after mitigating effects from the parental environment, suggesting that the response of that trait to the environmental gradient is genetically based in those populations. Our experiments with ancestral populations additionally showed that seed size is a heritable trait. Reciprocal common gardens are now needed to fully disclose the adaptive significance of seed size variation in ancestral populations (Blanquart et al. 2013). Gardens should be conducted along the elevation gradient with populations from low and high sites, and include measures of key fitness components, such as survival. Similarly, experimental explorations
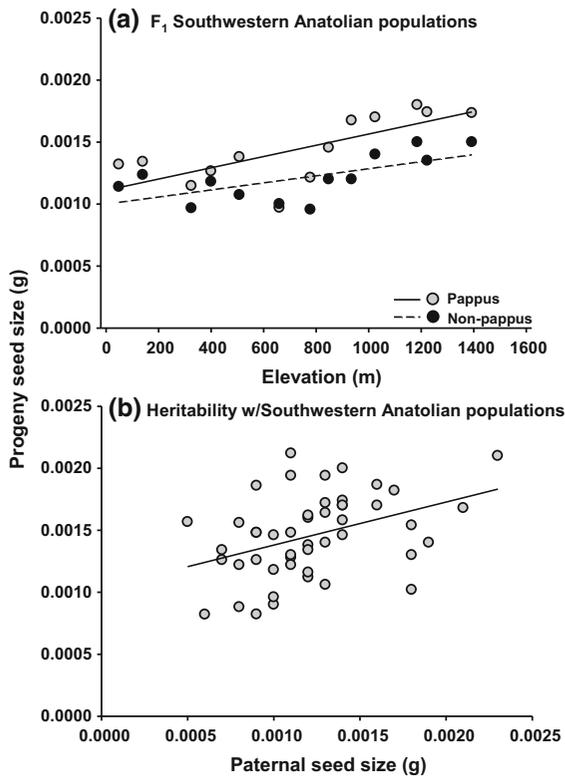
Fig. 4 a Seed size variation of ancestral populations grown under common conditions along an elevation gradient and **b** paternal-progeny relationship (heritability) for seed size in ancestral individuals of *Centaurea solstitialis*. In a) circles are means of four individuals per population (n = 13), except for one population with two individuals and two populations with three; in **b** circles are means of five pappus seeds per individual (n = 48)

along above lines are required to understand the plastic, adaptive, and/or pre-adaptive nature (Leger and Rice 2007; Alexander et al. 2009; MacDougall et al. 2018) of increments in seed size with elevation in expanded and non-native populations of *C. solstitialis*.

The purported genetic bases of seed size variation along the elevation gradient in ancestral populations indicates that a major differentiation event in that trait could have occurred within the ancestral range of *C. solstitialis*. Beyond that, mechanisms underlying the global pattern of seed size divergence reported here are currently unknown. Classical selection forces proposed to shape seed size, such as competition, predation, and dispersal (Rees and Westoby 1997; Buckley et al. 2003; Miguel et al. 2017), as well as plastic responses to the environment (Alexander et al. 2009) could be locally involved. Also, ancient and recent population admixture could have contributed to the pattern (Colautti and Lau 2015; Barker et al. 2017). In fact, our data are largely consistent with known dispersal routes (Eriksen et al. 2014) and the history of population admixture (Barker et al. 2017) of *C. solstitialis*. For example, Western European populations exhibited seeds intermediate in size between those in ancestral and non-native populations. Coincidently, Western European populations were the main source of American populations and the purported result of the admixture between ancestral and Eastern European populations. Dispersal pathways and evolutionary history seem thus to have played an
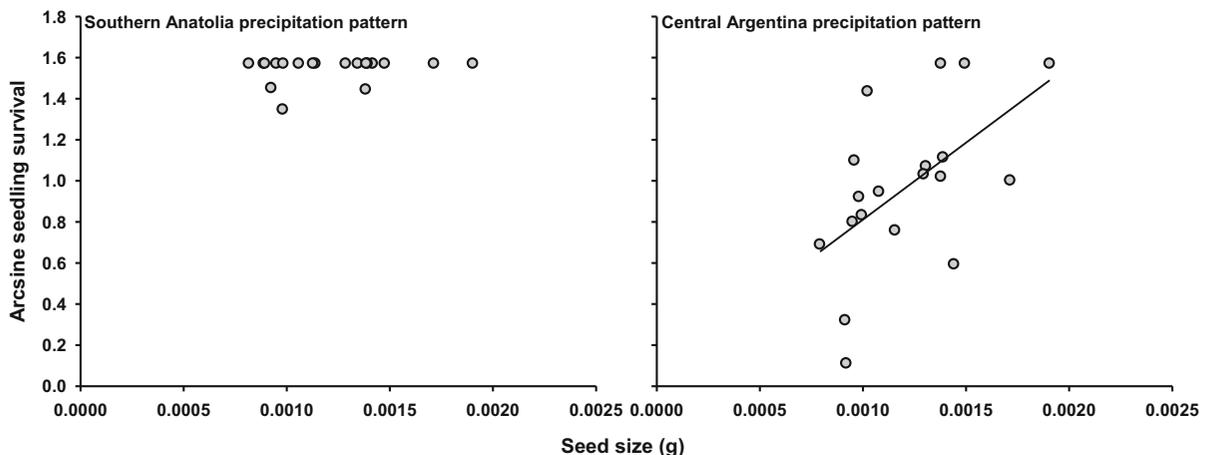

Fig. 5 The relationship between seed size and seedling survival in *Centaurea solstitialis* populations grown under simulated precipitation regimes in Aydin, Southwestern Anatolia, and Santa Rosa, Central Argentina. Circles are means of four pots

important role in shaping the current pattern of global seed size divergence in *C. solstitialis*.

Empirical studies have offered evidence both in favor (Leishman and Westoby 1994; Leishman et al. 2000; Caddick and Linder 2002; Peco et al. 2009) and against (Mazer 1989; Suárez-Vidal et al. 2017) theoretical expectations on a positive relationship between seed size and seedling survival contingent to environmental conditions (Venable and Brown 1988). As in previous work assessing demographic consequences of seed size divergence between ancestral and non-native populations of *C. solstitialis* in reciprocal common gardens in the field (Hierro et al. 2013), our current greenhouse data support the idea that seed size promotes plant fitness under unfavorable growing conditions (Venable and Brown 1988; Westoby et al. 1992; Moles and Westoby 2004). Large seeds could be critical for coping with the dry conditions commonly experienced by *C. solstitialis* at early life stages in Central Argentina, where the continental precipitation regime poorly matches the emergence time of the species (Hierro et al. 2009, 2013). Consequently, populations with large seeds could have been instrumental for colonizing Central Argentina. In addition, we found that seed size did not affect seedling survival under favorable growing conditions, which also fits theory (Venable and Brown 1988) and previous reports from field common gardens (Hierro et al. 2013). Importantly, however, our manipulation of precipitation regimes in the greenhouse allowed us to move one step further from field results, and offer evidence for the importance of seed size for the survival of *C. solstitialis* seedlings under the isolated influence of the precipitation pattern in Central Argentina.

Previous studies have also reported increments (Vera 1997; Blionis and Vokou 2002; Willis and Hulme 2004; Pluess et al. 2005; Alexander et al. 2009; Qi et al. 2015), decrements (Baker 1972; Totland and Birks 1996; Bu et al. 2007; Qi et al. 2015) and no changes (Pluess et al. 2005; Guo et al. 2010) in seed size with elevation. Those finding increments commonly propose that large seeds promote seedling survival under limiting conditions at high elevation. Knowledge of organismal responses to elevation gradients has traditionally been applied to understand responses to other environmental gradients, including latitude and continentality (Alexander et al. 2009; Pausas and Bond 2019). Similarly, that knowledge

may inform about responses to precipitation patterns in our work. That is, increased seed size in response to elevation in ancestral populations may have benefited the much later colonization of *C. solstitialis* populations in Argentina.

Gardens in the greenhouse additionally revealed that emergence rate can influence seedling survival under Central Argentinean conditions. These results are in line with those from germination experiments, in which *C. solstitialis* seeds in populations from Argentina exhibited lower germination proportions and rates than the rest of the worldwide assessed populations (Hierro et al. 2009). Also, germination proportions correlated with surrogates for environmental risk (i.e., inter-annual variation in winter precipitation and probability of favorable winters) across study regions (Hierro et al. 2009), suggesting that precipitation in Central Argentina may favor seed dormancy and slow germination/emergence. Populations of *C. solstitialis* in Argentina may thus posses a number of traits or an integrated suite of traits that confer them increased seedling survival under conditions in that region. The possibility of population differentiation in Central Argentina also deserves further exploration. Given that both ancestral and Western European populations seem to have contributed to founding populations in Argentina (Eriksen et al. 2014; Barker et al. 2017), Asian, European, and Argentinean populations should be integrated into those assessments.

Our study adds to a picture in which natural and human dispersal (Eriksen et al. 2014; Barker et al. 2017), ancient and recent admixture (Barker et al. 2017), response to local conditions (Hierro et al. 2009, 2013; Eriksen et al. 2014; Dlugosch et al. 2015; Barker et al. 2017; Miguel et al. 2017), and interruption of gene flow and subsequent divergence (Montesinos et al. 2012; Montesinos and Callaway 2017) could have influenced the colonization and range expansion of *C. solstitialis* around the world. Species globally dispersed by humans are thus powerful study systems where to address questions related to fundamental biogeographic processes.

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