

Rapid evolution of an invasive weed

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Summary

- Trade-offs between performance and the ability to tolerate abiotic and biotic stress have been suggested to explain both the success of invasive species and phenotypic differentiation between native and invasive populations. It is critical to sample broadly across both ranges and to account for latitudinal clines and maternal effects when testing this premise.
- Wild-collected *Centaurea diffusa* seeds were grown in benign and stressful conditions (drought, flooding, nutrient stress and simulated herbivory), to evaluate whether native and introduced individuals differ in performance or life history phenotypes. A second experiment used glasshouse-grown seeds to evaluate whether patterns remain comparable when maternal environment is consistent.
- Many traits differed between ranges, and in all cases but one, invasive individuals grew larger, performed better, or matured later. No trade-off in performance with herbivore defense was evident. Invasive populations may have been released from a trade-off between growth and drought tolerance apparent in the native range.
- Larger individuals with delayed maturity and greater reproductive potential have evolved in invasive populations, a pattern evident across broad population sampling, and after latitude and maternal environment were considered. Release from abiotic stress tolerance trade-offs may be important for the invasion success of *Centaurea diffusa*.

Introduction

Invasions represent excellent opportunities to synthesize evolution, ecology, and genetics over contemporary time-scales, and especially to address how human-induced changes in the environment and species distributions influence ecological and evolutionary processes. While extensive research focuses on the ecological processes that play a role in plant invasions, an understanding of the evolutionary processes involved is still maturing (Hendry *et al.*, 2008; Catford *et al.*, 2009; Buswell *et al.*, 2011). It is clear, however, that the success of an invasion may depend on adaptation to novel conditions, potentially many times, during the course of range expansion (Hufbauer *et al.*, 2012).

Such adaptation should be observable as ecologically important differentiation between populations from the native and invaded ranges. Increased growth rate or reproductive capacity is frequently reported from field observations in the invaded range (Elton, 1958; Crawley, 1987; Thébaud & Simberloff, 2001; Parker *et al.*, 2013), and increasingly from common garden experiments (reviewed in Felker-Quinn *et al.*, 2013). This improved vigor could contribute to rapid spread and population growth in the invaded range. Multiple hypotheses have attempted to explain increased performance of invasive populations, including the evolution of increased competitive ability hypothesis (EICA; Blossey & Notzold, 1995). EICA posits that selection will favor genotypes with reduced allocation to herbivore defense

and increased allocation to growth, reproductive output, or competitive ability in the absence of herbivores characteristic of the native range (Blossey & Notzold, 1995). EICA thus predicts that, as a consequence of trade-offs, individuals from the native range will produce less biomass than individuals from the introduced range, and specialist herbivores will show improved performance on individuals from the introduced range in a common environment (Blossey & Notzold, 1995; Joshi & Vrieling, 2005). EICA is only supported if increases in growth are linked to decreases in defense. Though increased performance in invasive individuals relative to native is often observed in common garden experiments, shifts in defenses are less common (Kumschick *et al.*, 2013), and thus evidence for EICA is equivocal (Felker-Quinn *et al.*, 2013). Some authors have expanded ideas about trade-offs to include tolerance to other types of stress, not just herbivore pressure (Bossdorf *et al.*, 2005; He *et al.*, 2010). If novel habitats are less stressful than native ones, selection would favor individuals that shift resources from stress tolerance to increased vigor and fecundity. Invoking trade-offs assumes that plants are unable both to be highly tolerant to stressful environments and to be highly competitive or to have high reproductive output (Grime, 1977). Several species demonstrate such trade-offs. For example, tolerance to an abiotic stress, such as serpentine soils or drought, comes at the expense of competitive ability or growth rate (Sambatti & Rice, 2007). EICA and other trade-off hypotheses suggest that invasive individuals evolve a lower tolerance to biotic

or abiotic stress, and therefore will perform relatively poorly under stressful conditions, as assessed in several studies (e.g. Hodgins & Rieseberg, 2011; Lachmuth *et al.*, 2011; Kumschick *et al.*, 2013). However, trade-offs can occur in multiple directions (e.g. Burton *et al.*, 2010). Additionally, strategies favored by selection may change over time, between different phases of an invasion (Dietz & Edwards, 2006) or depending on the habitats invaded (Lachmuth *et al.*, 2011). A shift in resource allocation under benign conditions is not the only explanation for increased growth and reproduction in a species' invaded range. Other genetically based changes between the native and invaded ranges that could lead to increased performance include inter- or intra-specific hybridization (Rieseberg *et al.*, 2007; Schierenbeck & Ellstrand, 2009; Lai *et al.*, 2012).

Observing the phenotypes of a single generation in a common environment may be insufficient to demonstrate adaptation to a novel habitat. Latitudinal clines can impede our ability to infer evolutionary change from common gardens (Colautti *et al.*, 2009). Even within the invaded range of a species, local adaptation can vary along latitudinal and environmental clines as the invading populations adjust to local environments, shifting phenology, biomass, and other trait means (Colautti *et al.*, 2010; Lachmuth *et al.*, 2011). It is also necessary to rule out differences between the native and invaded ranges caused by maternal environmental effects. Maternal effects can have strong, even adaptive, impacts on offspring phenotype in some systems (Galloway, 2005). Yet their influence in shaping performance differences between ranges is only rarely experimentally controlled (except see Monty *et al.*, 2009; Hodgins & Rieseberg, 2011).

To test trade-off hypotheses, and to look for evidence of rapid adaptation to novel habitats, we ask: (1) Do native and invasive populations show consistent phenotypic differences in growth and reproduction, and do such differences remain even after controlling for latitude and maternal effects? and (2) Is there evidence of a trade-off between the ability to grow quickly under benign conditions and the ability to tolerate stressful conditions? To address these questions, we conducted two large glasshouse experiments with *Centaurea diffusa* (diffuse knapweed), one of North America's worst weedy invaders (Lejeune & Seastedt, 2001). The first experiment, hereafter the broad CG, included 28 native European and 18 invasive North American populations of *C. diffusa* (Fig. 1), using one of the highest levels of population replication in studies of this type (see also Kumschick *et al.*, 2013). The second experiment, hereafter the maternal CG, assessed whether patterns observed in the broad common garden were maintained after controlling the maternal environment by using seeds produced from glasshouse crosses of four populations from each range. Both gardens included a benign control treatment and biotic and abiotic stress treatments (simulated herbivory, nutrient deficiency, drought, and flooding). Differences between the two experiments would suggest that the maternal environment (as well as experimental variation) exerts a significant influence on phenotypic response. Similarity between them would suggest that genetic polymorphisms control the phenotypic divergence between native and invasive individuals (Moloney *et al.*, 2009). Furthermore, if plants from the invaded range outperform plants from the native range in the control treatment, but not in the stress treatments, hypotheses invoking trade-offs would be supported. However, a lack of significant

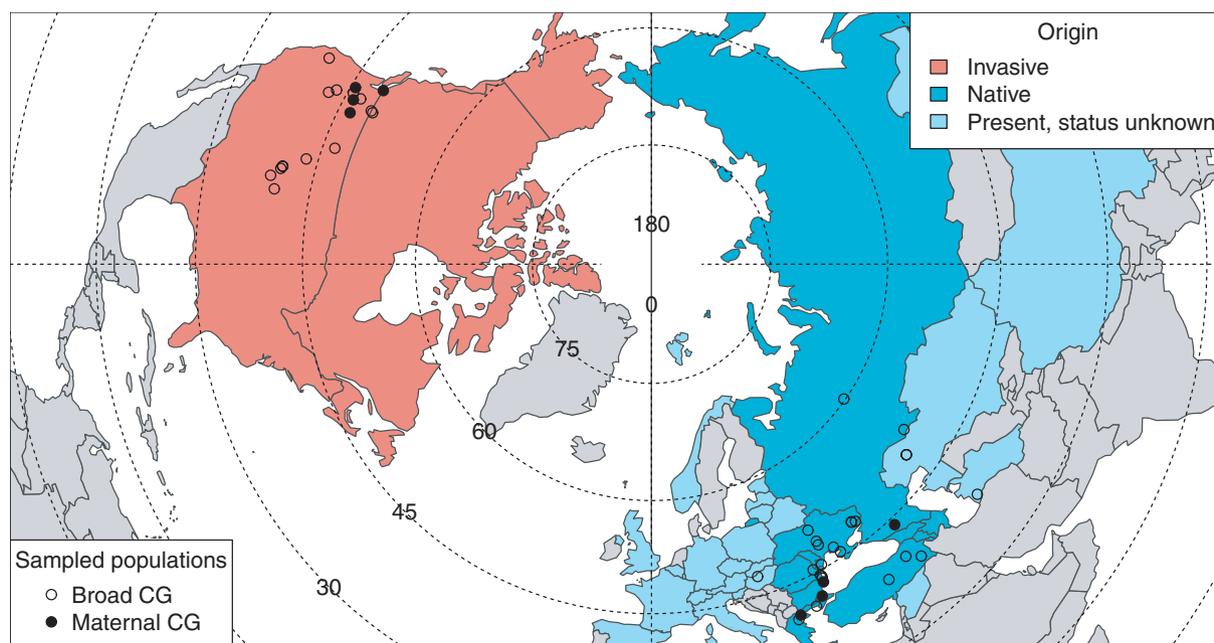


Fig. 1 Range and collection map for *Centaurea diffusa* in the Northern Hemisphere, by country. Populations used in the various experiments are indicated by point style; all populations were used in the broad common garden (open circles), and four from each origin in both broad and maternal common gardens (filled circles). Origin status in a particular country is indicated by color, and was determined from the classification in Greuter (2006), ISSG.org (2013), Kartesz (2013), Tropicos.org (2013), and USDA (2013). 'Present, status unknown' also includes countries where *Centaurea diffusa* is considered naturalized. Degrees of latitude are indicated on dotted lines, and degrees of longitude are indicated on solid lines. CG, common garden experiment.

differences between the two ranges may indicate that *C. diffusa* success in North America does not rely upon evolutionary differences between ranges.

Materials and Methods

Study species

Centaurea is the most abundant noxious weed genus in the western USA, and along with its congeners, diffuse knapweed (*Centaurea diffusa* Lam.) reduces the quality of forage for livestock and wildlife, and alters soil and water resource availability (Lejeune & Seastedt, 2001). *Centaurea diffusa* is a monocarpic biennial (Thompson & Stout, 1991), native to eastern Europe and western Asia, and now found throughout western Europe as well (Fig. 1; Greuter, 2006). It was first reported in North America in 1907 in Washington State (Sheley *et al.*, 1998) and now occurs in nearly half of the USA and Canada (Fig. 1; USDA, 2013). Field surveys of *C. diffusa* reveal differing impacts of herbivores between ranges, with North American plants experiencing more seedhead herbivory, and European plants experiencing more root herbivory (Blair *et al.*, 2008). The seedhead weevil, *Larinus minutus* (Col.: Curculionidae), introduced in the late 1990s, is the biological control agent associated with the largest decline in density of knapweed species, including *C. diffusa* (Seastedt *et al.*, 2005; Myers *et al.*, 2009).

Population genetic surveys indicate that *C. diffusa* was introduced to North America multiple times and harbors comparable levels of diversity at molecular loci to the native range (Hufbauer & Sforza, 2008; Marrs *et al.*, 2008). *Centaurea diffusa* can hybridize with diploid spotted knapweed, *Centaurea stoebe* ssp. *stoebe* L. (Blair & Hufbauer, 2009, 2010; Blair *et al.*, 2012; Lai *et al.*, 2012). The tetraploid form of spotted knapweed (*Centaurea stoebe* ssp. *micranthos* (Gugler) Hayek, sometimes referred to as *Centaurea maculosa*) is a successful invader of North America, but the diploid form does not occur there (Treier *et al.*, 2009; Blair & Hufbauer, 2010; H. Müller-Schärer pers. comm.). Hybridization between the diploids has been confirmed by both amplified fragment length polymorphism and transcriptome comparisons (Blair *et al.*, 2012; Lai *et al.*, 2012), which indicate that hybrid ancestry occurs in approximately one-quarter of North American *C. diffusa* individuals (Blair & Hufbauer, 2010), and that the genomic extent of introgression may be greater in introduced than native individuals (Lai *et al.*, 2012). These data suggest that highly introgressed genotypes of *C. diffusa* colonized North America.

Seed collections

Seeds were collected in a broad collaborative effort from 30 native European populations and 27 invasive North American populations of *C. diffusa* from 2001 to 2008. Samples with sufficient germination (46 populations) were included in the broad CG (Fig. 1). Some populations had lower germination success, and so were excluded from some analyses (Supporting Information Tables S1,S2).

Broad common garden

Centaurea diffusa plants from field-collected seeds were grown under benign or stressful conditions in a glasshouse at the University of British Columbia. Seeds were germinated on filter paper in 1% plant preservative mixture and distilled H₂O at room temperature. Within 12 d, c. 1500 seedlings, from three to six families from each population, were transplanted into 5-cm-diameter cones filled with 80% potting mix and 20% silica sand.

Seedlings were randomly assigned to four stress treatments and a control group. Within each treatment, populations were represented by at least three families. In total, 498 invasive and 1047 native plants were included. Each stress treatment contained c. 215 plants, and the control group contained c. 400 plants. A subset of plants were destructively sampled at the onset of the stress treatments (213). Within a treatment, c. 35 plants were randomly assigned to a position within each block. Blocks were randomly ordered across a glasshouse bench, and rotated randomly each week. Supplemental lighting maintained a 16-h day.

Treatments began at 6 wk (plants had an average of 10 leaves larger than 3 cm), and included three abiotic stresses (nutrient deficiency, drought, and flooding) and one biotic stress (simulated herbivory). Plants were watered with fertilizer until the treatments began, when all plants except those in the nutrient stress treatment were top-dressed with Osmocote 13-13-13 (The Scotts Company LLC, Marysville, OH, USA) and wand-watered from above. Drought blocks were no longer watered, and flood blocks were placed in individual bins that were filled with water to just above soil level, maintained by daily top-ups, and changed weekly. Plants in drought and flooding treatments probably experienced differences in soil nutrient availability relative to the control group. However, we were interested in comparing the relative responses of native and invasive populations rather than isolating responses to particular stresses.

In the herbivory treatment, the apical half of every other leaf of each rosette was cut, eliminating at least one-quarter of the plants' aboveground biomass. The plant was then sprayed with 1 mM methyl jasmonate, a plant hormone induced by insect herbivory (Norton *et al.*, 2008). The following week, remaining leaves were cut, and each subsequent week, new leaves over a length of 3 cm were cut, each time immediately followed by a methyl jasmonate spray. This occurred weekly for the first 5 wk of treatment when growth was rapid, then every other week as growth slowed.

Morphometric measurements were taken before treatments began (5 wk after germination) and before harvest (16–18 wk), and included shoot mass, root mass, root crown diameter, area of longest leaf, and number of basal leaves (Table S3). Phenological and survival traits were assessed every 2–3 d throughout the experiment, and included bolting status, bolting date, date of first stress response (wilting and yellowing), date of advanced stress response (total wilting and root death) and date of death. Responses to flooding were scored as follows. Roots were defined as dead when the plant detached from its soil, and shoots when all basal leaves detached from the stem under gentle pressure. At harvest, root biomass was washed to remove debris, and then

whole roots and shoots were harvested, dried at 29°C, and weighed.

We conducted leaf choice trials using *L. minutus*, a specialist weevil which feeds on floral and leaf tissue of *C. diffusa* (Stephens & Myers, 2013). Recently emerged adults were collected from three sites in British Columbia (Table S2), where *L. minutus* was introduced in 2000 (Myers *et al.*, 2009). Weevils were collected several times, and held for < 5 d before trials. Leaf material was taken from herbivory treatment plants sampled immediately before the first treatment, and then 1 wk later, and thus these plants could express both constitutive and induced defenses. For each trial, a single weevil was placed in a well with a moist cotton pad and allowed to acclimate for at least 1 h. Fresh cut leaf discs 6.3 mm in diameter were placed on the cotton pad, one each from an invasive and a native plant. The weevil was then placed between the two leaf discs and left to eat until one was half eaten or for up to 6 h. Leaf discs were frozen and then scanned to quantify tissue consumption using IMAGEJ 1.45s (Rasband, 2011). Each invasive population was represented in six trials paired with a sample from three to five randomly chosen native populations.

Maternal common garden

To rule out maternal environmental effects as a cause for differences observed between native and invasive populations, glass-house-generated seeds were used in the second experiment. Plants used to produce seeds for the maternal CG were from eight populations in the broad CG (Fig. 1, Tables S1,S2), and were grown at the same time and under the same conditions as the broad CG. Two individuals from each of four or five families for each population were transplanted into 1-l pots containing potting

soil. Plants that flowered between November 2009 and March 2010 were repeatedly reciprocally crossed with all individuals flowering simultaneously in the same population. Flower heads were bagged before anthesis and after hand pollination to prevent cross-fertilization. Seeds from these crosses were used in the maternal CG. For each family, germination traits were tracked for 10 d (overall average, *c.* 2 d to germinate).

In this experiment, treatments and measurements were the same as in the broad CG. In total, this garden included 230 plants, equally divided between native and invasive populations. In each stress treatment, each population was represented by three individuals (24 plants total) from different sets of parents when possible. The control treatment included 111 plants, and the early harvest control included 23 plants. Phenotypic differences between native and invasive individuals were assessed as in the broad CG, except for the following. First, morphometric measurements were taken slightly later (before stress treatment, 15 leaves on average, 7 wk after germination; and before harvest, 19–20 wk). Secondly, additional leaves were sampled at harvest, one mature leaf to determine specific leaf area, and one young leaf for future genomic work. Thirdly, root biomass was not measured. Finally, leaf choice trials were expanded to include four time-points (one constitutive and three induced), each 1 wk apart, and *c.* 350 additional trials run using every individual from the invasive populations.

Statistical analysis

To determine if *C. diffusa* differs phenotypically between its native and invasive ranges, we compared morphological and life history traits between these two ranges. Using R 3.0.1 (R Core

Table 1 Selected examples of means and confidence intervals estimated from range differentiation models of phenotypes of *Centaurea diffusa* grown in both common garden experiments

Trait	Distribution and model	Origin				P
		Native		Invasive		
		Estimate	CI	Estimate	CI	
Broad common garden						
Control						
No. of basal leaves	Poisson, REML	12.43	11.04–13.99	16.28	15.31–17.32	***
Bolting status (harvest) (%)	Binomial, REML	24.30	17.49–32.69	2.07	0.62–6.76	[**]
Herbivory						
Root mass (g)	Gaussian (\log_e), REML	1.03	0.88–1.21	1.37	1.09–1.72	*
Bolting status (harvest) (%)	Binomial, GLM	26.71	20.17–34.47	6.45	2.44–15.97	[***]
Maternal common garden						
Control						
No. of basal leaves	Poisson, REML	11.74	8.51–16.21	20.41	14.81–28.14	*
Bolting status (harvest) (%)	Binomial, GLM	75.09	54.53–88.34	7.57	2.51–20.67	[*]
Nutrient						
Area of longest leaf (cm ²)	Gaussian, GLM	56.45	41.76–71.13	82.74	68.05–97.42	**
Herbivory						
Bolting status (harvest) (%)	Binomial, GLM	84.70	36.30–98.18	0.44	0.01–24.82	[**]

CI, 95% confidence interval; \log_e , natural log transformation of data.

Least squares (LS) means are given from restricted maximum likelihood (REML) models including origin and all significant terms (interactions were excluded). Where no random effects were significant, LS means were estimated from generalized linear models (GLMs). The significance of the origin term (or origin \times latitude in square brackets []) is indicated in the right-most column: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Team, 2013), we employed restricted maximum likelihood (REML) models with random effects using the LME4 package. Univariate response traits were modeled separately for each treatment. Because nondestructive early control measurements took place before treatments were initiated, they included both control and stress treatment plants, under control conditions. Gaussian distributions were fitted for continuous measures and natural log-transformed when necessary to improve normality of residuals (Tables 1,S4,S5). Poisson distributions were fitted for count data and binomial distributions were fitted for binary data.

To assess the significance of each model term, we removed each term or interaction in a stepwise manner based on likelihood ratio tests (LRTs). The χ^2 test statistic, degrees of freedom, and *P*-values reported are from these LRTs (Tables 2,S3, S6). When random effects were nonsignificant, LRTs were run using generalized linear models (GLMs) without random effects, and the results of these LRTs are reported. All non-Gaussian minimal GLMs were checked for over-dispersion. Because the effect of one variable depends on the condition of the other, it is not meaningful to test the significance of main effects that are included in significant interactions during step-wise model simplification (Crawley, 2012) and so in some cases these are not reported. Least squares (LS) means are reported as a measure of effect size, and are estimated from models including all significant terms except interactions (Tables 1,S4,S5).

In range differentiation models, origin (native or invasive), latitude, and their interaction were included in all full models. Population (uniquely named), maternal lines nested within population, and population variance within each origin were used as random effects in all full models (e.g. trait ~ origin × latitude + (origin|population/maternal line)). Additional models were

run to explicitly test for the existence of trade-offs in stress treatments. In these trade-off models, a population mean measure of performance in the benign control treatment (population mean shoot mass) and its interaction with origin were included in models of stress tolerance traits, along with all of the terms included in the range differentiation models. A significant interaction indicates a different relationship between performances under benign and stressful conditions between the two origins. When a trade-off model had a significant interaction, slopes of regression lines from model estimates are reported (Table S7).

Some mixed model analyses combined data from both experiments. To maximize statistical power, data from the leaf choice trials of both gardens were analyzed together (Table S8), including generation as a random effect. In these models, the ‘generation’ term includes maternal effects as well as experimental variation between gardens. Additionally, to explicitly test for similarity between the two gardens, origin, generation, and their interaction were included as fixed effects in models of several traits under control conditions from the populations and lines included in the maternal CG (Table 3). Other fixed and random effects were included as described for range differentiation models.

Results

Broad common garden

For 11 of the 31 traits measured across all treatment levels and time-points, native and invasive individuals exhibited different phenotypes, demonstrated by a significant origin or origin × latitude term. For every measure of size with a significant difference between the ranges, invasive individuals were larger (Fig. 2a,b).

Table 2 Test statistics from range differentiation models for selected phenotypic traits of *Centaurea diffusa*

Trait	Fixed effects			Random effects		
	Origin χ^2 (df) <i>P</i>	Latitude χ^2 (df) <i>P</i>	Origin × latitude χ^2 (df) <i>P</i>	Populations χ^2 (df) <i>P</i>	Maternal lines χ^2 (df) <i>P</i>	Populations within each origin χ^2 (df) <i>P</i>
Broad common garden						
Control						
No. of basal leaves	13.08 (1)***	5.91(1)*	1.50 (1)	66.22 (1)***	257.29 (3)***	37.14 (4)***
Bolting status (harvest)	nt	nt	7.14 (1)**	0.57 (1)	10.71 (1)***	4.07 (4)
Herbivory						
Root mass	4.33 (1)*	15.82 (1)***	0.06 (1)	13.04 (1)***	<0.01 (1)	0.84 (4)
Bolting status (harvest)	nt	nt	14.46 (1)***	<0.01 (1)	0.32 (1)	6.24 (4)
Maternal common garden						
Control						
No. of basal leaves	4.20 (1)*	0.64 (1)	3.75 (1)*	4.48 (1)*	215.7 (1)***	4.22 (4)
Bolting status (harvest)	nt	nt	5.78 (1)*	<0.01 (1)	0.11 (1)	0.68 (4)
Nutrient						
Area of longest leaf	6.89 (1)**	1.12 (1)	1.32 (1)	0.13 (1)	<0.01 (1)	1.47 (4)
Herbivory						
Bolting status (harvest)	nt	nt	7.34 (1)**	<0.01 (1)	<0.01 (1)	<0.01 (4)

df, degrees of freedom; χ^2 , chi-squared test statistic; nt, not tested, because of significant interaction term.

The effect of origin and latitude on phenotypes of *Centaurea diffusa* grown in a common environment are reported for both the broad and maternal common gardens. Results are presented from restricted maximum likelihood (REML) models. Where no random effects were significant, generalized linear models (GLMs) were used to test fixed effects. The significance of a term is indicated by symbols: *, *P* < 0.1; **, *P* < 0.05; ***, *P* < 0.01; ****, *P* < 0.001.

Table 3 Effect of origin and generation on phenotypes of *Centaurea diffusa* grown in a common garden, across two generations

Trait	Fixed effects			Random effects		
	Origin χ^2 (df) <i>P</i>	Generation χ^2 (df) <i>P</i>	Origin \times generation χ^2 (df) <i>P</i>	Populations χ^2 (df) <i>P</i>	Maternal lines χ^2 (df) <i>P</i>	Populations within origin χ^2 (df) <i>P</i>
Early control						
No. of basal leaves	0.32 (1)	44.72 (1)***	0.79 (1)	2.47 (1)	1.29 (1)	0.59 (4)
Control						
Shoot mass	0.77 (1)	7.92 (1)**	0.06 (1)	13.35 (1)***	0.76 (1)	0.91 (4)
Area of longest leaf	7.93 (1)**	41.97 (1)***	0.02 (1)	1.30 (1)	<0.01 (1)	0.26 (4)
No. of basal leaves	nt	nt	15.10 (1)***	19.61 (1)***	185.07 (3)***	11.72 (4)*
Bolting status (harvest)	46.33 (1)***	1.80 (1)	0.22 (1)	1.29 (1)	2.40 (1)	0.04 (4)
Bolting date	nt	nt	8.21 (1)**	2.05 (1)	69.19 (1)***	1.21 (4)

df, degrees of freedom; χ^2 , chi-squared test statistic; nt, not tested, because of significant interaction term.

Results are presented from restricted maximum likelihood (REML) models. Where no random effects were significant, generalized linear models (GLMs) were used to test fixed effects. The significance of a term is indicated by symbols: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

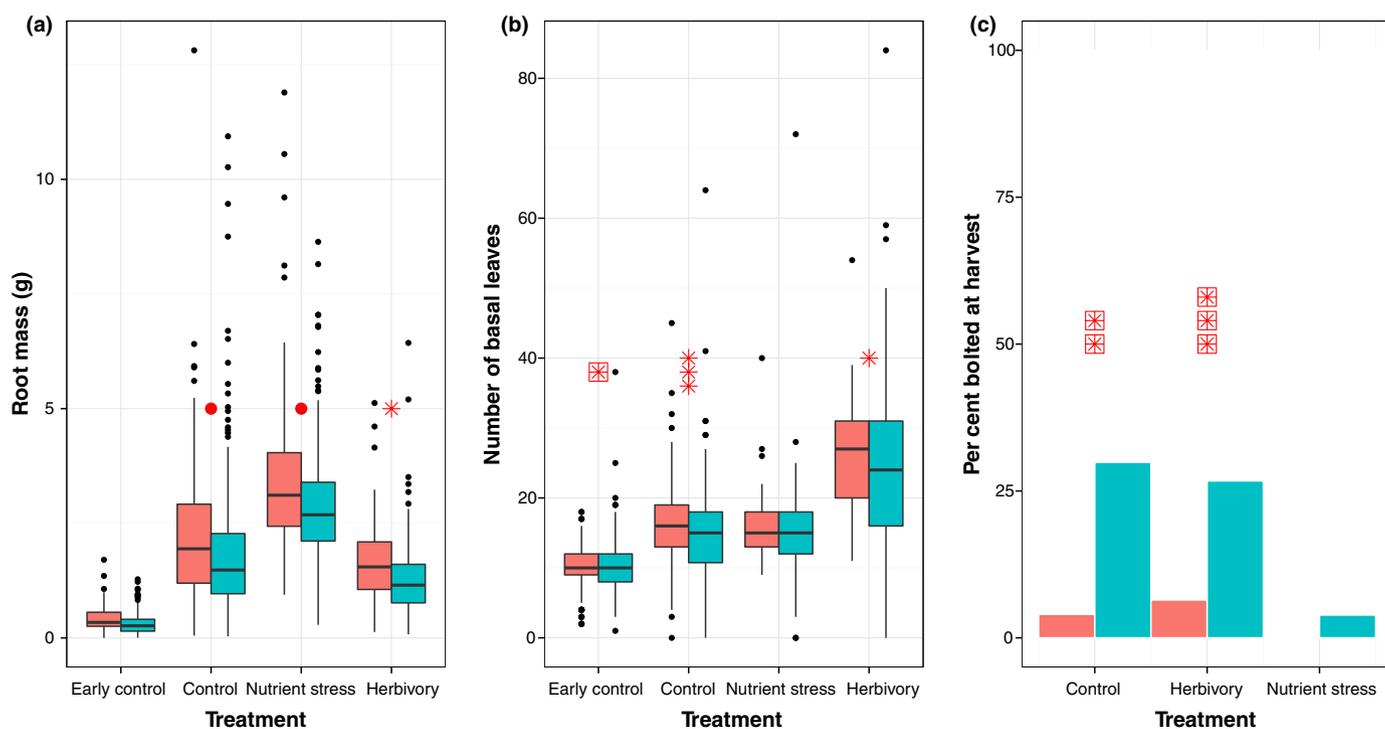


Fig. 2 Phenotypic difference between ranges of *Centaurea diffusa* individuals that survived until harvest in the broad common garden for the control and all stress treatments. Origin: pink, invasive; blue, native. Significance level of origin (or origin \times latitude in box) in range differentiation models (Table 2) is indicated by red asterisks and large red dots: •, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Sample size by treatment: early control, varies by trait; control, invasive = 125; native = 261; nutrient, invasive = 81; native = 180; herbivory, invasive = 62; native = 146. Two size traits at harvest are shown; (a) root mass, and (b) number of basal leaves. The early life stage control is included for comparison. Early control sample size: for root mass, invasive = 54; native = 145; for leaf number, invasive = 498; native = 1047. Whiskers extend from the median to the highest and lowest values within $1.5 \times$ the interquartile range. (c) Proportion of individuals that bolted by harvest.

Life history also differentiated the ranges: when significant, invasive individuals matured later (i.e. bolted; Fig. 2c). Latitude was a significant covariate for most traits (19 traits), though rarely had a significant interaction with origin (three traits). Significant random effects were common, especially population (20 traits). Seed weight and seed collection age were not significantly different between native and invasive mothers.

Under benign control conditions, latitude was a significant covariate, or had a significant interaction with origin, for most

traits (Table S3). Invasive individuals matured later than native individuals (Fig. 2c, Table 2); at the time of harvest, 24% of native individuals had bolted, while only 2% of invasive individuals had bolted (Table 1). Origin \times latitude was significant for this trait such that in native populations, increasing latitude marginally decreased the proportion of bolted individuals within a population, while in invasive populations, the proportion bolted did not vary with latitude (Tables 2, S9). Root crown diameter (a measure of developmental stage) did not predict bolting, in contrast to

Powell & Myers (1988) (root crown diameter, $\chi^2 = 0.48$; $P = 0.487$). Few invasive individuals had bolted by harvest, making the analysis of bolting date problematic, although it showed a similar trend. Invasive individuals also had approximately three more basal leaves at harvest than natives (Fig. 2b). Origin \times latitude was significant for this trait; leaf number increased with latitude, but more steeply in invasive than native populations (Tables 2, S9). Origin was marginally significant for two additional size traits (root crown diameter at *c.* 6 wk, and root mass at harvest; Table 2), and, in each case, invasive individuals were larger. The remaining traits showed no effect of origin (Table S3).

Under nutrient-deficient conditions, both shoot and root masses exhibited a marginally significant difference between the ranges (Fig. 2a). Invasive individuals had more below-ground and above-ground biomass than native individuals (Table S4). The difference in overall trait means between this stress condition and the control condition (overall mean root mass was *c.* 3.1 g for nutrient stress and 2.0 g for control) indicates that this treatment was sufficient to cause a response in plant biomass.

Under drought conditions, invasive plants wilted significantly faster than native plants, although there was no effect of origin on time to total wilt or death in range differentiation models (Table S3). Trade-off models of these drought tolerance traits revealed a significant interaction between origin and performance in the benign control in each case (Fig. 3a, Table 4). For all three traits, the pattern is the same. Native populations exhibit a trade-off between drought tolerance and size in benign conditions; populations that produce larger individuals in benign conditions are less tolerant to drought. Invasive populations appear to have lost this trade-off; they may be less tolerant than the natives (origin significant only for days to first wilt in range differentiation models), but this tolerance does not decrease with size in the broad CG in any of the traits measured, and even marginally increases with size in days to total wilt (Table S7).

Under flood conditions, there was no effect of origin on any trait in range differentiation models. However, in trade-off models, there was a significant interaction between origin and control performance in days to death. For both native and invasive populations, populations that produced larger plants in benign conditions survived longer under flooding conditions; however, larger size was significantly more advantageous in invasive populations (Fig. 3b, Tables 3, S7).

In the herbivory treatment, origin had a significant effect on both the size and life history of the plants in range differentiation models (Fig. 2, Table 2). Invasive individuals had more root mass than native individuals and the difference in overall trait means between this treatment and the control (*c.* 1.4 g for simulated herbivory and 2.0 g for control) indicates that this stress was sufficient to cause a response in biomass. As in the control, more native individuals had bolted by the end of the experiment than invasive individuals (27% of native versus 6% of invasive individuals; Table 1). Origin \times latitude was significant for this trait, because plants bolted in only the three northernmost invasive populations. In the native populations, the likelihood of bolting did not vary with latitude (Table S9).

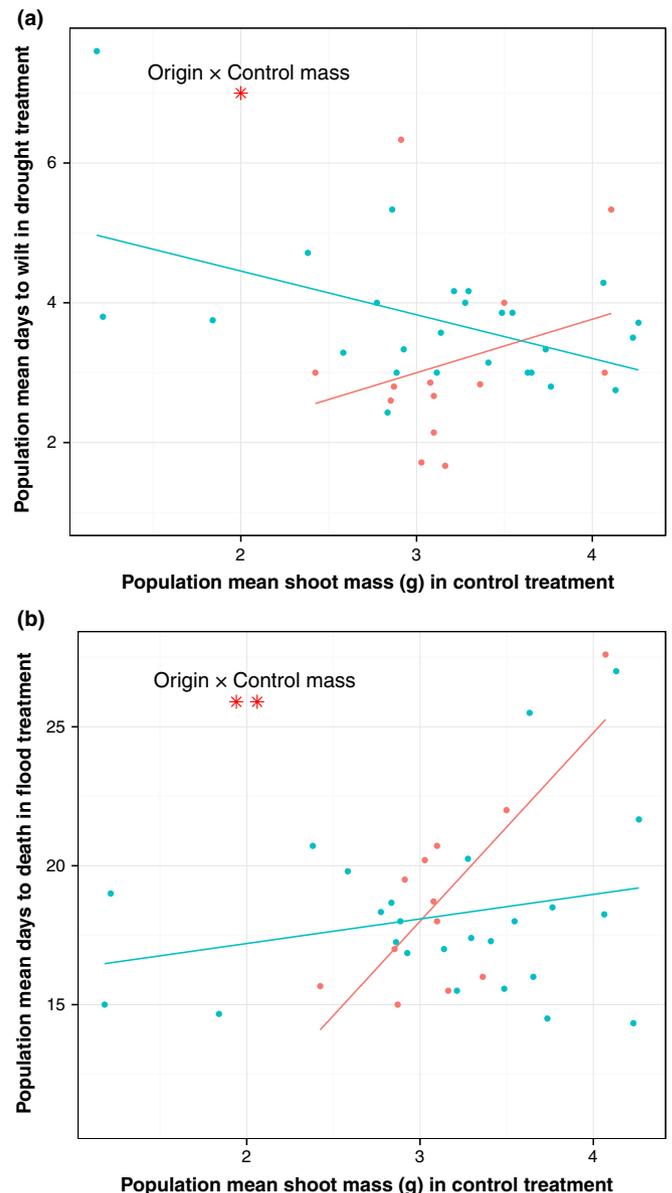


Fig. 3 Variation in stress tolerance and performance in benign conditions between ranges of *Centaurea diffusa* in the broad common garden. Points represent population means. Origin: pink, invasive; blue, native. Text and asterisks indicate the significance level of origin \times control mass (population mean shoot mass in control treatment) in trade-off model analyses (Table 4); red asterisks: *, $P < 0.05$; **, $P < 0.01$. (a) Days to first wilt in drought treatment (invasive = 13 populations (65 individuals); native = 26 populations (145 individuals)). (b) Days to death in flood treatment (invasive = 12 populations (49 individuals); native = 25 populations (122 individuals)).

Maternal common garden

Native and invasive phenotypes were different or marginally different for 13 of the 27 traits measured across all treatment levels and time-points (Table S6), demonstrated by a significant origin or origin \times latitude term. As before, for every measure of size with a significant difference between the ranges, invasive individuals were larger (Fig. 4a, Table S5). Life history traits again differentiated the ranges: when significant, invasive individuals

Table 4 Explicit trade-off models of phenotypes of *Centaurea diffusa* grown in stress conditions in both common garden experiments

Trait	Fixed effects				Random effects	
	Origin χ^2 (df) <i>P</i>	Control mass χ^2 (df) <i>P</i>	Origin \times control mass χ^2 (df) <i>P</i>	Latitude χ^2 (df) <i>P</i>	Populations χ^2 (df) <i>P</i>	Maternal lines χ^2 (df) <i>P</i>
Broad common garden						
Drought						
Date of 1st wilt	nt	nt	4.84 (1)*	3.31 (1)*	2.33 (1)	<0.01 (1)
Date of total wilt	nt	nt	4.17 (1)*	5.99 (1)*	2.83 (1)*	<0.01 (1)
Death date	nt	nt	4.36 (1)*	3.55 (1)*	<0.01 (1)	<0.01 (1)
Flood						
Death date	nt	nt	9.89 (1)**	4.80 (1)*	4.51 (1)*	4.35 (1)*
Maternal common garden						
Drought						
Date of 1st wilt	2.10 (1)	0.88 (1)	1.31 (1)	0.48 (1)	<0.01 (1)	<0.01 (1)
Date of total wilt	nt	nt	5.63 (1)*	0.31 (1)	<0.01 (1)	<0.01 (1)
Death date	0.60 (1)	0.46 (1)	0.03 (1)	1.75 (1)	<0.01 (1)	<0.01 (1)
Flood						
Death date	0.01 (1)	1.27 (1)	0.33 (1)	0.17 (1)	<0.01 (1)	<0.01 (1)

df, degrees of freedom; χ^2 , chi-squared test statistic; nt, not tested, because of significant interaction term.

Test statistics are reported for the interaction between stress tolerance phenotypes and performance in benign control conditions ('control mass', or the population mean shoot mass in the control treatment) for *Centaurea diffusa* grown in a common environment, for both the broad and maternal common gardens. The three-way interaction and the random effect of population variation within each origin were also tested, but were never significant. Results are presented from restricted maximum likelihood (REML) models. Where no random effects were significant, generalized linear models (GLMs) were used to test fixed effects. The significance of a term is indicated by symbols: •, $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$.

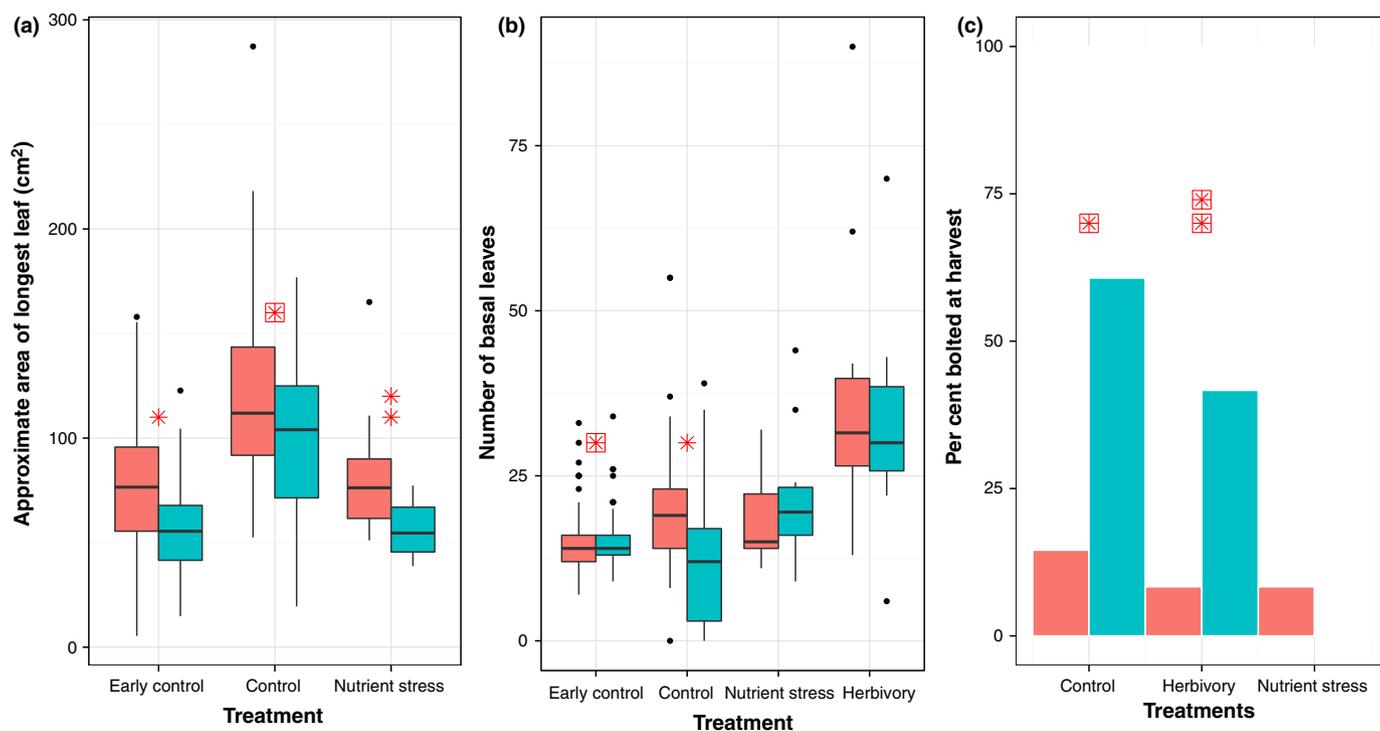


Fig. 4 Phenotypic difference between ranges of *Centaurea diffusa* individuals that survived until harvest in the maternal common garden for the control and all stress treatments. Origin: pink, invasive; blue, native. The significance level of origin (or origin \times latitude in box) in range differentiation models (Table 2) is indicated by red asterisks: *, $P < 0.05$; **, $P < 0.01$. Sample sizes by treatment was as follows: early control, invasive = 115; native = 115; control, invasive = 55; native = 56; herbivory, invasive = 12; native = 12; nutrient, invasive = 12; native = 12. Two size traits at harvest are shown; (a) approximate area of longest leaf and (b) number of basal leaves. The early life stage control is included for comparison. Whiskers extend from the median to the highest and lowest values within $1.5 \times$ the interquartile range. The herbivory treatment is not represented in the leaf size measure because leaf biomass was experimentally altered. (c) Proportion of individuals that bolted by harvest.

matured later (Fig. 4c). Origin \times latitude was significant for all of the same traits, and one additional trait (four traits total). However, unlike the previous garden, latitude was rarely a significant covariate (one trait). Because of the subset of populations available for use in the maternal CG, latitude and origin are statistically confounded; the latitudinal ranges do not overlap (40°–44° native and 45°–49° invasive). However, there is no reason to expect that origin and latitude are biologically confounded (i.e. that phenotypes differ between ranges simply because one range is more southerly than the other), as general results are congruent with the broad CG, where these two terms are not confounded. For some traits, a significant effect of latitude may be obscured by the confounded origin term, although latitude is still important for several traits (seven traits). Random effects were less common than in the previous garden, and population was significant for only four traits.

From intra-population crosses in the glasshouse, invasive individuals produced more seeds, and had a higher germination rate, than their native counterparts (Table S6). Origin had a significant effect on germination date in the maternal CG; invasive seeds germinated earlier than native seeds. Seed weight did not exhibit an effect of origin.

Under control conditions, most size traits showed a significant effect of origin or origin \times latitude in the maternal CG (Fig. 4a,b; Table S6). As in the previous garden, invasive individuals had more basal leaves at harvest than native individuals (Tables 1, 2). Origin \times latitude was significant for number of basal leaves at 7 wk; number of basal leaves increased with latitude among native individuals but marginally decreased with latitude among invasive individuals (Table S9). Additionally, invasive individuals were larger than natives for several measures: area of longest leaf and shoot mass at 7 wk and shoot mass at harvest. Area of longest leaf at harvest exhibited a significant effect of origin \times latitude; area increased with latitude among native individuals, and decreased with latitude among invasive individuals (Table S9). Bolting status at harvest again showed a significant effect of origin \times latitude. At harvest, 67% more native individuals had bolted than invasive individuals (Fig. 4c, Table 1). As in the broad CG, the likelihood of bolting in invasive populations did not vary with latitude. However, for natives the trend reversed; likelihood of bolting increased with latitude (Table S9). Among plants that bolted, invasive individuals bolted later, although the significance of origin in this model was marginal.

Under nutrient stress, leaves of invasive individuals were nearly 50% larger than native leaves (Tables 2,S5). No trait measured in the drought condition showed a significant difference between native and invasive individuals in range differentiation models. However, in the trade-off model of days to first wilt, there was a significant interaction between origin and control performance, although only for this trait (Table 4). As before (Fig. 3a), native populations demonstrated a trade-off between size in the control treatment and drought tolerance that was not apparent in the invasive populations. Invasive drought tolerance even increased with size in one trait (days to total wilt) and a similar trend was seen in another (days to first

wilt; Table S7). Origin was not significant for any trait in the flood treatment in range differentiation models. No significant interactions were evident in trade-off models of the flood treatment, although the trend for days to death was similar as in the broad CG (Fig. 3b). In the herbivory treatment, bolting status at harvest had a significant effect of origin \times latitude (Fig. 4c). For invasive populations, the likelihood of bolting did not vary with latitude, and for native populations, it may have increased with latitude, although, because only three of eight populations included bolting individuals in this treatment, this result should be treated with caution (Table S7).

Cross-generational analyses

Analysis of the combined leaf choice trial data from both gardens showed no difference between origins or experiments for either preference or amount consumed, and weevils' preference was evenly divided (Table S8). Generation was not a significant random effect in these models. There was a significantly negative effect of week on the amount of leaf disc eaten by the weevils, among the post-stress time-points, suggesting a cumulative effect of increased herbivore defense and decreased palatability in response to the treatment.

A subset of traits measured in control conditions in both experiments were explicitly examined for the effect of generation (i.e. maternal effects as well as experimental variation between gardens). Differences were common; of the six traits analyzed in this way, only one showed no effect of generation (Table 3). Origin \times generation was significant for two traits: number of basal leaves at harvest significantly increased with generation in invasive populations, while it decreased in native populations, and for bolting date, invasive populations did not significantly vary with generation, while native populations bolted earlier after a generation in the glasshouse (Fig. 5). For all other traits, origin either remained significant as in the two gardens separately, or gained significance. Latitude was not included in this analysis because it is confounded with origin for these populations.

Discussion

Evolution in the invaded range

These experiments reveal genetically based phenotypic differences in growth and reproduction between the native and invaded ranges of *C. diffusa* (Question 1). Under benign common conditions, *C. diffusa* demonstrates life history trait differences and greater growth and reproductive potential in the invaded compared with the native range, although populations in these ranges are separated by little more than 100 yr. Invasive individuals are larger than natives, and when maternal effects are controlled for, this effect is even more apparent.

Invasive individuals also have delayed maturity, suggesting an adaptation to a longer growing season, or a shift toward decreased use of the facultative annual strategy. Such a shift could position individuals to optimally utilize seasonal resources, such as precipitation. Delayed maturity could also have strong impacts on the

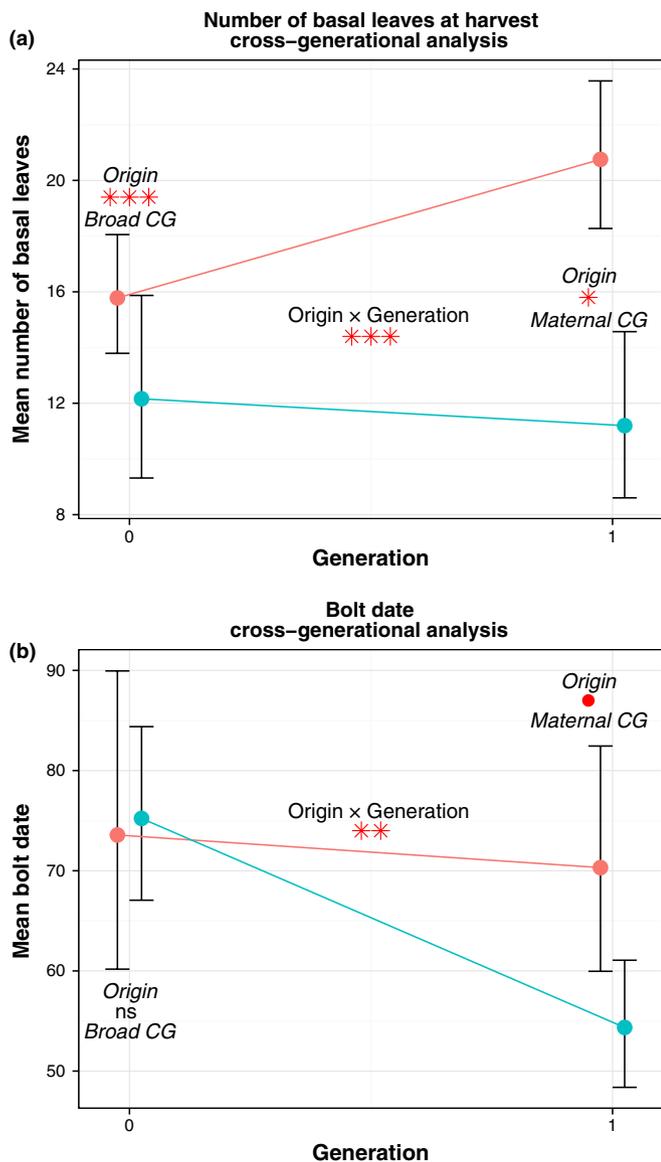


Fig. 5 Phenotypic differences between ranges of *Centaurea diffusa* across generations, for four native (blue) and four invasive (pink) populations in the control treatment. Data presented are only from these eight populations. The significance level of origin \times generation in range differentiation models (Tables 2,3) indicated by text, red asterisks, and large red dots: ns, not significant; *, $P < 0.1$; **, $P < 0.05$; ***, $P < 0.01$; ****, $P < 0.001$. The text in italics is there as a reminder of the significance (or not) of origin in mixed model analyses from the broad common garden (generation 0; model including all populations in that experiment), the maternal common garden (generation 1), and combined data sets (middle). Bars represent 95% confidence intervals from mixed model analyses. (a) Number of basal leaves at harvest (invasive = 96; native = 91, in combined data set). (b) Bolting date, for the subset of plants that bolted before harvest (invasive = 11; native = 52, in combined data set). CG = common garden experiment.

invasion success of these populations; if delayed bolting means the plant is larger at flowering, then it has the potential to produce more flower heads, more seeds, and more progeny. This potential for increased fitness is demonstrated from the results of our glasshouse crosses: the larger invasive individuals produced more seeds than their native counterparts. Under nutrient-

limited conditions in both gardens, minimal bolting occurred (Figs 2c, 4c), suggesting that overall the species is not adapted to low-nutrient conditions and that adaptation to this environment does not vary between ranges, a pattern consistent with other characteristics of opportunistic species. In the face of human-induced selective pressures and novel environments (Hufbauer *et al.*, 2012), *C. diffusa* has demonstrated the capacity to rapidly adapt, which may have contributed to its successful invasion of North America.

Performance traits showed a significant effect of origin across broad population sampling, after an attempt to control for maternal environment, and even, in some cases, across stressful treatment environments. Origin frequently had a significant effect on phenotype, even when latitude was included in statistical models, indicating that differences in phenotype are not attributable solely to latitude, a concern raised by Colautti *et al.* (2009). Broad patterns of growth and life history were consistent across both gardens. These patterns also held in the combined data set, a balanced data set that minimized maternal effects, and allowed verification of results after a generation in a common environment. When differences in maternal effects were minimized, some traits gained a significant effect of origin, while others lost this effect; this difference may be attributable to the maternal environment influencing results in the broad CG. However, our seed collections covered a broad geographic and latitudinal range, no sampling biases between ranges for collection date are apparent (Fig. S1), and seed weight, which is commonly used as a proxy for maternal effects, did not vary between the ranges (Table S3). The reduction of maternal environmental 'noise' in the maternal CG may have allowed more traits to show a significant effect of origin in the control treatment. But, while this experiment may have decreased the effects of the maternal environment, as a result of limited population and seed sample size, some analyses may have lacked power to detect differences that were significant in the broad CG. Insufficient power may be a concern in the stress treatments, each of which only contained 24 individuals. Other potential explanations for differences between the two gardens include the inadvertent reduction of genetic variation by crossing only a subset of plants from each population, or more environmental variation occurring over the course of one experiment than the other.

The focus of this study on broad sampling and controlling maternal environment may have allowed us to detect range variation not seen in previous studies of this species (Blumenthal & Hufbauer, 2007; Blair *et al.*, 2012). Other aspects may affect the analysis of our data. Population relatedness has been shown to have a strong effect on the analysis of phenotypic variation in common gardens, and the implications of this variation for adaptive evolution (Lachmuth *et al.*, 2011); however, it was not directly assessed here. Population genetic analyses of these populations are ongoing. Also, growth or biomass production measured without competition may not translate to performance (particularly competitive ability) in the field (see Vilà & Weiner, 2004; Gruntman *et al.*, 2013). Blair *et al.* (2012) compared the performances of native and introduced *C. diffusa* experiencing competition from a North American

native grass in a glasshouse, and found no effects of range. Additionally, further analysis of the differences in abiotic environment between the two ranges is needed to elucidate the relationship between invasiveness and abiotic stress tolerance in this species, and may have more explanatory value than latitude alone.

Test of EICA and trade-off hypotheses

Investigations into trade-offs in *C. diffusa* reveal a trade-off in performance between plant growth in a benign environment and tolerance to some stresses (Question 2) in the native, but not the invaded range. In the case of herbivory, none of the predictions of the EICA hypothesis were supported. Invasive individuals did as well as or better than natives in both benign control and simulated herbivory treatments. Palatability was not significantly different between the ranges in the leaf choice trials, suggesting no defense trade-off against a specialist herbivore in the invaded range, a pattern also seen with a generalist herbivore by Blair *et al.* (2008). A possible caveat is that the specialist used has been present as a biological control agent at some locations in the invaded range for up to 15 yr – this brief re-exposure may be enough to select for renewed herbivore defense in some populations. Weevil and seed collections overlapped at only two locations (Table S2); however, *L. minutus* is widespread in North America (R.A. Huffbauer, pers. obs.). A recent meta-analysis of experiments testing EICA found evidence for genetically based changes between the native and introduced ranges in defense, growth, and competitive traits (Felker-Quinn *et al.*, 2013), but not necessarily in the direction predicted by EICA, and without evidence for trade-offs between growth and defense against herbivores. Our results here are consistent with this conclusion.

Our results do, however, provide some evidence for trade-offs between growth and tolerance to abiotic stressors, including both drought and flooding, between the two ranges in this species. Under drought stress, native populations exhibited a trade-off between drought tolerance and size in benign conditions – populations that produced smaller plants were more drought tolerant. The invasive populations appear to have lost this trade-off, as tolerance to drought did not decrease with size in either experiment in any of the three traits measured, and even increased with size in days to total wilt in the maternal CG. However, it is interesting to note that they were less tolerant than the natives overall, at least in terms of the first signs of wilt in the broad CG. The evidence for a flood tolerance trade-off is less well supported, but suggestive. In the broad CG, for both native and invasive populations, populations that produced larger plants in benign conditions took longer to die under flooding conditions; however, this trade-off was significantly steeper for invasive populations. In the maternal CG, the trend for this trait was similar, but nonsignificant. Taken together, these results suggest the prevalence of ‘all-purpose genotypes’ (Baker, 1965), which do well in a wide array of environments, in invasive populations. These ‘all-purpose genotypes’ may be released from the constraint apparent in the native range, where native populations may produce large individuals or tolerant individuals but not both. There is some

evidence that invasive populations have the potential to produce both very large and very tolerant individuals.

Other explanations are possible for these data. For example, controlled glasshouse conditions are not the same as those experienced by the plant in its habitat, and may fail to reveal important interactions observable in field conditions, especially nutrient–water interactions. Genetically based changes between the native and invaded ranges could include the effect of inter- or intra-specific hybridization, and has been documented in *C. diffusa* (Blair *et al.*, 2012; Lai *et al.*, 2012). Hybridization is proposed to stimulate invasiveness through the generation of novel genotypes – by recombination, heterosis, increased fitness, and decreased genetic load (Schierenbeck & Ellstrand, 2009). In several species, intra-specific genetic admixture has resulted in higher genetic diversity in invasive compared with native populations, hybrid vigor, and loss of inbreeding depression (Kolbe *et al.*, 2004; Keller & Taylor, 2010; Verhoeven *et al.*, 2011). Hybridization may even result in stress-tolerant ‘all-purpose genotypes’ (Baker, 1965). While a causal link between invasion and hybridization has been demonstrated rigorously in some cases (Facon *et al.*, 2005; Lavergne & Molofsky, 2007), it is generally not clear if hybridization is a cause or consequence of range expansion, or if it has an impact on ‘invasiveness’ *per se*. However, because this study does not explicitly address the genetic background of the populations involved, further work is required to establish the impact of inter- or intra-specific hybridization in this species.

Conclusions

Our data suggest that post-invasion evolution may have played a significant role in the invasion success of *C. diffusa* in North America, but do not rule out the impact of pre-invasion hybridization with *C. stoebe* ssp. *stoebe*. Field surveys of *C. diffusa* in its native and invaded ranges found larger, more robust plants in North America (Blair & Huffbauer, 2009). As Blair *et al.* (2011) suggest, some of these differences probably result from variation in environmental field conditions. Our data indicate, however, that variation in the maternal environment associated with field-collected seeds may confound common garden studies involving *C. diffusa*. We controlled for effect of latitude, and minimized maternal effects by producing seeds under controlled environmental conditions, revealing evidence for post-invasion evolution. Our data do not follow all of the necessary predictions of EICA; however, they do lend support to trade-offs for some abiotic stress tolerances, especially drought tolerance, in the native but not the invasive range. Invasive populations appear to have lost some variation in drought tolerance, which may limit what types of climate they can invade, or alternately invasive populations may avoid drought as a consequence of their extended life cycle. While the genetic basis for these patterns is currently unknown, future common garden and genetic work will explore the possibility that intra- or inter-specific hybridization has resulted in the prevalence of ‘all-purpose genotypes’ in the invaded range and aim to identify the genetic changes that enabled *C. diffusa* to become one of the most successful weedy invaders of western North America.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Germination results for field-collected *Centaurea diffusa* seeds.

Table S1 Native *Centaurea diffusa* population information

Table S2 Invasive *Centaurea diffusa* population information

Table S3 Test statistics for all traits measured in the broad common garden, from range differentiation models of phenotypes of *Centaurea diffusa* grown in a common garden

Table S4 Means and confidence intervals for all traits estimated from range differentiation models of phenotypes of *Centaurea diffusa* grown in a common garden

Table S5 Means and confidence intervals for all traits estimated from range differentiation models of phenotypes of *Centaurea diffusa* grown in a common garden, after one generation

Table S6 Test statistics for all traits measured in the maternal common garden from range differentiation models of phenotypes of *Centaurea diffusa* grown in a common garden, after one generation in the glasshouse

Table S7 Z ratios and parameter estimates from explicit trade-off models for *Centaurea diffusa* grown in a common garden which included a significant interaction between origin and control treatment performance (control shoot mass)

Table S8 Effect of origin, latitude, and constitutive defense on herbivore preference of *Centaurea diffusa* grown in a common garden, across two generations

Table S9 Z ratios and parameter estimates from range differentiation models of *Centaurea diffusa* grown in a common garden which included a significant interaction between origin and latitude

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