POPULATION ECOLOGY - ORIGINAL PAPER

Different gardens, different results: native and introduced populations exhibit contrasting phenotypes across common gardens

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Abstract Invasive plants may respond through adaptive evolution and/or phenotypic plasticity to new environmental conditions where they are introduced. Although many studies have focused on evolution of invaders particularly in the context of testing the evolution of increased competitive ability (EICA) hypothesis, few consistent patterns have emerged. Many tests of the EICA hypothesis have been performed in only one environment; such assessments may be misleading if plants that perform one way at a particular site respond differently across sites. Single common garden tests ignore the potential for important contributions of both genetic and environmental factors to affect plant phenotype. Using a widespread invader in North America, *Cynoglossum officinale*, we established reciprocal common gardens in the native range (Europe) and introduced range (North America) to assess genetically based differences in size, fecundity, flowering phenology and threshold flowering size between native and introduced genotypes as well as the magnitude of plasticity in these traits. In addition, we grew plants at three nutrient levels in a pot experiment in one garden to test for plasticity across a different set of conditions. We did not find significant genetically based differences between native and introduced populations in the traits we measured; in our experiments, introduced populations of *C. officinale*

were larger and more fecund, but only in common garden experiments in the native range. We found substantial population-level plasticity for size, fecundity and date of first flowering, with plants performing better in a garden in Germany than in Montana. Differentiation of native populations in the magnitude of plasticity was much stronger than that of introduced populations, suggesting an important role for founder effects. We did not detect evidence of an evolutionary change in threshold flowering size. Our study demonstrates that detecting genetically based differences in traits may require measuring plant responses to more than one environment.

Keywords *Cynoglossum officinale* ·
Phenotypic plasticity · Evolution of increased competitive ability (EICA) hypothesis · Founder effects ·
Native and introduced ranges

Communicated by Rebecca Irwin.

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Introduction

Plants adapted to particular conditions in their home range are increasingly introduced into new areas, where conditions may differ. How exotic species cope with these novel environmental conditions in recipient communities is an area of growing interest in ecology (Blossey and Nötzold 1995; Hänfling and Kollman 2002; Maron et al. 2004; Sakai et al. 2001; Stockwell et al. 2003). Some have suggested that the lag time between the initial introduction and resulting spread of an invader might be the result of plants evolving adaptations to these new conditions (Byers et al. 2002; Lee 2002). A growing number of studies have tested this hypothesis and have found evidence for genetically based changes in phenotype in common gardens (Blair and Wolfe 2004; Bossdorf et al. 2004, 2005; Joshi and Vrieling

2005; Leger and Rice 2003; Maron et al. 2004; Siemann and Rogers 2003; Stastny et al. 2005; van Kleunen and Schmid 2003; Wolfe et al. 2004). The hypothesis that motivated most of these studies, proposed by Blossey and Nötzold (1995), is that exotic plants released from their specialist natural enemies in the introduced range might be selected to reallocate energy away from producing costly defenses toward increased growth or reproduction (the evolution of increased competitive ability (EICA) hypothesis). Such an evolutionary switch in energy allocation might give plants a competitive advantage in the introduced range. However, to date, results from tests of this hypothesis have been mixed. Some studies find that individuals are larger in introduced populations or that defenses are lower, others find the opposite result, and some studies have found no pattern at all (reviewed in Bossdorf et al. 2005).

A challenge in interpreting the results of tests of the EICA hypothesis is that, typically, plants are grown in only one common environment. For example, of the 26 studies that have compared phenotypes between native and introduced populations in common gardens (reviewed by Bossdorf et al. 2005), only five utilized common gardens in more than one environment and only two of those had common gardens in both the native and introduced ranges. Since Bossdorf et al. (2005), 18 additional EICA tests have been published, of which only three were performed in more than one common garden (Genton et al. 2005; Maron et al. 2007; Widmer et al. 2007). The use of only one garden can present problems in interpretation if there are substantial differences in phenotypic plasticity among ranges of origin, i.e. if there are genotype by environment interactions. For example, imagine the situation in which plants collected from introduced populations outperform those from native populations in one common garden, but the reverse is true in another common garden. In this case, data from only one garden might lead one to ascribe differences in performance between native and introduced populations wholly to genetically controlled shifts in plant phenotype, whereas in actuality, phenotypic differences between gardens would indicate a large genotype by environment interaction.

The potential problem of using only one common garden can be further exacerbated if there are large founder effects among introduced populations. Again, imagine the example where exotic genotypes outperform native genotypes of the same species in a single common environment. In this case, this result might be due to the fact that introduced populations were founded by a relatively small number of native genotypes. These introduced genotypes could have originated from a restricted set of native locales where they were adapted to local environmental conditions. If these original environmental conditions happen to be similar to those in the chosen common garden site, then these genotypes might outperform native genotypes. Because native genotypes may come from a greater diversity of populations, some of which experience very different climatic conditions than the garden site, on average, native populations might underperform introduced populations.

To help alleviate these issues, we performed a reciprocal common garden experiment in the native and introduced ranges to compare levels of fixed and plastic differences in phenotype among native and introduced populations of a widespread invasive plant of western North America, houndstongue (*Cynoglossum officinale* L. Boraginaceae). Here we describe experiments where we have used one common garden in each range (in Montana and Germany), but if the logistical challenges could be overcome, having more than one garden in each range would lend greater insight into the strength of genotype by environment interactions. In addition to field garden experiments, we also explicitly manipulated growing conditions (soil nutrient levels) in an outdoor pot experiment in the native range to further explore the magnitude of fixed versus plastic responses in the traits we measured in larger gardens, and to also determine whether threshold flowering size in this semelparous plant has increased in introduced populations. We use results from both the reciprocal field common garden and nutrient addition (pot) experiments to ask: have plant size, fecundity, date of initial flowering, and average plasticity for these three traits increased in populations of *C. officinale* between the native and introduced ranges? Furthermore, in the nutrient addition experiment: has the median threshold flowering size increased between native and introduced populations?

We quantified levels of plasticity among native and introduced genotypes across gardens because it has recently been proposed that selection should favor the evolution of greater plasticity among introduced populations (Richards et al. 2006). Although comparing average levels of plasticity for particular traits between native and exotic genotypes appears straightforward, in practice it can present difficulties. The traditional approach to estimating phenotypic plasticity has been to compare the response of genetically related individuals across multiple sites (Pigliucci 2001). However, in the case of natives versus exotics, replicating genotypes at the individual, genetic family, population, and regional (native vs. introduced range) levels requires a number of samples that becomes logistically problematic. One solution to this, which we have adopted here, is to compare average differences in plasticity among native and introduced populations, where there are replicate individuals within each population, but not replicate individuals within replicate families within each population. This approach, while less precise than the

traditional methods for estimating plasticity, can still be appropriate for comparing native and introduced populations (Muth and Pigliucci 2007; Richards et al. 2006). It is also necessitated, because any comparison of native and introduced phenotypes requires sampling genotypes from a sufficient set of populations across each range to ensure a representative sample of native and introduced genotypes. Only a few studies have explicitly tested for increased phenotypic plasticity using populations from both ranges (DeWalt et al. 2004; Kaufman and Smouse 2001; Maron et al. 2007; Muth and Pigliucci 2007; Bossdorf et al. in Richards et al. 2006).

We measured threshold flowering size to test the life history prediction that relative growth rate and the probability of mortality before reproduction dictate the optimal threshold size for flowering (Roff 1992; Wesselingh et al. 1997). If the probability of pre-reproductive mortality decreases in the introduced range, potentially due to escape from enemies, increased threshold flowering size between native and introduced populations might evolve.

Materials and methods

Houndstongue, *C. officinale* L. (Boraginaceae), is native to Europe, where it grows in disturbed sites, open woodlands, meadows and sand dunes (de Jong et al. 1990). Its native range extends from the mountains of western Asia and eastern Europe west to the Netherlands, and north to southern Britain and Scandinavia; it is not present in the southern Mediterranean regions of Europe (de Jong et al. 1990). It was first introduced to North America in the mid-19th century as a feed contaminant and is now present across the USA and southern Canada, where it is particularly common in forest clearcuts and overgrazed rangelands (Upadhyaya et al. 1988). It is classified as a noxious weed in six western states, where it occurs at high density and is toxic to cattle and horses (Upadhyaya et al. 1988).

Cynoglossum officinale is a self-compatible, facultatively biennial forb (de Jong et al. 1990) that forms a rosette in its first year after germinating in the early spring, overwinters as a rosette and taproot, and then bolts and flowers in the summer of its second or later year, depending on plant size and environmental conditions. Whether or not plants flower at the end of their second summer depends on individuals attaining a threshold flowering size (de Jong et al. 1998), which is both environmentally and genetically determined (Wesselingh et al. 1997). Each flower produces fruits at the end of the summer consisting of up to four large nutlets. Plants invest all of their stored energy into seed production and then die, with vegetative size prior to flowering positively and highly correlated with seed production (de Jong and Klinkhamer 1988).

A specialist root-boring weevil, *Mogulones cruciger*, that is present only in the native range, preferentially attacks large rosettes and flowering plants, and can reduce seed set (Prins et al. 1992). In the native range, *C. officinale* is also attacked by a specialist stem-boring weevil and two leaf-feeding flea beetles (Schwarzlaender 2000; M. Schwarzlaender, personal communication). These specialists are not present in the introduced range, where herbivory by generalists such as Lepidopteron larvae and grasshoppers does not affect plant size or fecundity (J. Williams, unpublished data).

Field common gardens in the native and introduced ranges

We established common gardens in Missoula, Montana and Bad Lauchstädt, Germany (environmental conditions described in Table 2). The soil was tilled in both gardens in March 2004 prior to planting. In Montana, we applied the herbicide Roundup two weeks before tilling to remove existing weeds. We quantified soil nitrogen and carbon from ten bulk soil samples (collected with a 3 cm diameter soil borer to a depth of 10 cm) from each garden in April 2006, at the conclusion of the experiment. Soil was sieved through 2 mm mesh and then ground in a Wiley mill using a 20 M screen. All samples were analyzed in a CN-Analyzer for %N, %C, C/N ratio and pH. Differences in mean values between the two gardens were evaluated using t-tests that assumed unequal variance between groups. Both gardens were fenced to keep out animals. In Germany, specialist root boring and leaf chewing insects (*Mogulones cruciger* and *Longitarsus* spp., respectively) were not present in the garden.

In 2003, we collected seeds from ten *C. officinale* populations in the native range (Europe) and introduced range (North America), respectively (Table 1). Seeds from each population were collected from 10–15 individuals, separated by at least 1 m. Ten maternal seed sources were randomly selected from each source population and seeds were put into cold stratification for 6 weeks starting in December 2003 to break seed dormancy. We planted seeds into small pots in greenhouses in Missoula, Montana and Bad Lauchstädt, Germany in early February 2004. Seeds were sown in a 1:1 mixture of compost and sand.

We planted the seedlings into the gardens in Germany on 1 April 2004 and in Montana on 18 April 2004. Each common garden was divided into ten blocks, with one plant from each family randomly assigned to block, for a total of 200 plants per garden (two continents × ten populations × ten maternal families). Every plant in each garden had a sib in the other garden. In Montana, plants within blocks were spaced 0.75 m apart, with blocks separated by 1 m. In Germany, due to space constraints, plants within a

Table 1 Conditions in common gardens: Germany garden in Bad Lauchstädt, Saxony-Anhalt, and Montana garden in Missoula, Montana

	Germany garden	Montana garden
Mean annual rainfall (mm)	484	351
Mean January high temperature (°C)	4.0	−0.7
Mean January low temperature (°C)	−0.6	−8.8
Mean July high temperature (°C)	23.9	28.7
Mean July low temperature (°C)	13.8	19.4
Percent soil nitrogen	0.18 ± 0.01	0.35 ± 0.02**
Percent soil carbon	2.46 ± 0.30	4.10 ± 0.23**
Soil carbon/nitrogen ratio	13.58 ± 0.85	11.66 ± 0.06*
Soil pH (measured in water)	7.56 ± 0.06	6.81 ± 0.04**

Bad Lauchstädt climate data from UFZ Department of Soil Physics working group "C/N Dynamics" and Missoula climate data from US National Weather Service, Missoula station; long-term averages reported for both gardens. Soil properties are reported with one SE of the mean. Significant differences in soil properties between gardens denoted as ** for $P < 0.001$ and * for marginal significance, $0.05 < P < 0.10$

block were spaced 0.5 m apart, with 0.9 m separating blocks. Seedlings were watered on the initial planting date, after which they received only ambient rainfall.

We quantified date of first flowering by recording the approximate day that the first flower completely opened on each plant; gardens were visited 2–3 times per week during the period of initial flowering. We assessed plant size at the end of the first growing season in fall 2004 by measuring the diameter and height of each rosette and calculating plant volume using the equation for a cylinder. The vast majority of plants in both gardens began flowering in spring 2005 and we harvested all plants after they had set seed in July 2005, but before plants died and released their seeds. In the Montana garden, we directly counted all seeds produced by each plant. In Germany, the plants were too large to count every seed. We therefore estimated fecundity by multiplying the number of inflorescences (cymes) on each plant by the average number of seeds per cyme. We estimated the average number of seeds per cyme by counting the number of seeds on each of 20 randomly selected cymes.

Nutrient experiment

To experimentally determine how variation in resource availability influences plant size, fecundity, date of first flowering and threshold flowering size, we also established an experiment where we manipulated fertilizer levels to create three different nutrient treatments. Seedlings were planted in 1 liter pots with a mixture of, by volume, 67% washed sand and 33% compost soil ("La Terra") and transferred to the experimental garden in Bad Lauchstädt, Germany, on 3 May 2004. Due to logistical constraints, we were only able to perform this experiment in one location. Pots were placed in experimental beds filled with bark mulch to protect them from extreme temperatures. The low

Table 2 Source populations of *Cynoglossum officinale* seeds used in common gardens

Continent	State/Country	Collection site	Latitude and longitude
North America	Wyoming	Afton	42°43′N; 110°58′W
North America	Montana	Boulder River	45°39′N; 110°06′W
North America	Montana	Livingston	45°43′N; 110°28′W
North America	Washington	Clarkston	46°25′N; 117°03′W
North America	Idaho	Dworshak Resevoir	46°42′N; 116°17′W
North America	Montana	Ninemile Prairie	46°57′N; 113°32′W
North America	Montana	Lavalle Creek	46°58′N; 114°04′W
North America	Montana	Tamarack Creek	47°21′N; 115°03′W
North America	British Columbia	Fenwick Road	49°33′N; 115°32′W
North America	Alberta	Pincher Creek	49°44′N; 114°02′W
Europe	Hungary	Cobex	46°28′N; 020°25′E
Europe	Hungary	Korduskut	46°30′N; 020°40′E
Europe	Germany	Aseleben	51°28′N; 011°41′E
Europe	Germany	Salziger See	51°29′N; 011°44′E
Europe	Germany	Lettewitz	51°34′N; 011°50′E
Europe	Germany	Hohenerxleben 1	51°51′N; 011°38′E
Europe	Germany	Hohenerxleben 2	51°50′N; 011°37′E
Europe	Netherlands	Bierlap	52°08′N; 004°21′E
Europe	Netherlands	Meijendel Dunes	52°09′N; 004°20′E
Europe	Germany	Neustrelitz	54°22′N; 013°05′E

nutrient treatment received no additional fertilizer, the medium nutrient treatment received half of the recommended dosage (3 g) and the high nutrient treatment received the recommended dosage of 6 g of slow-release fertilizer (Osmocote 8–9 M). Six seed families from each of the 20 populations (ten from the native range and ten from the introduced range) were randomly chosen for this experiment, as we did not have enough space to use all ten maternal families from each population. We planted one seedling from each family into each fertilizer treatment, so that each replicate consisted of three nutrient levels with one sib at each level. All plants in the nutrient experiment were watered when necessary, because the sand in the small pots dried out quickly. We assessed plant size at the end of the growing season in 2004 and date of first flowering and fecundity in 2005, using the same methods described above for the German common garden.

Statistical analyses

We used analyses of variance (ANOVAs) to examine differences between plants from the native and introduced ranges in plant volume, fecundity and date of first flowering for both experiments. We first ran one analysis to examine overall differences in these three traits, where we treated location of garden (Germany or Montana), range (native or introduced) and the interaction of garden × range as fixed factors, and population nested within range and garden × population nested within range as random factors (Proc GLM in SAS, SAS 9.1, SAS Institute, 2003). Since each garden represented a different environment, a significant main effect of garden indicates plasticity for that trait. A significant interaction between garden and range indicates that the magnitude of the plastic response is dependent on the range of population origin. We report the magnitude of plasticity for each range as the percent increase in the trait ([(trait$_{Germany}$ − trait$_{Montana}$)/trait$_{Germany}$] × 100), calculated for each population and then averaged within range. Here we are considering plasticity at the population level, as an average across individuals from each population (Maron et al. 2007; Neubert and Caswell 2000; Richards et al. 2006), rather than in the strict sense, of at the genotype level. We used Tukey post hoc tests to test for significant differences in traits between native and introduced populations in each garden.

To test for among population differences in plasticity, we ran analyses separately for native and introduced populations. We treated garden as a fixed factor and population and garden × population as random factors (Proc GLM, SAS). A significant garden by population interaction indicates that populations within a continent vary in plasticity.

In the nutrient addition experiment, we used ANOVAs to examine both genetically based and plastic differences in the three traits we measured. Here, we treated nutrient level (low, medium or high), range (native or introduced) and nutrient level × range as fixed factors and population nested within range and nutrient level × population(range) as random factors (Proc GLM, SAS).

To assess threshold flowering size, we used a logistic regression to examine the effect of size in 2004 and range (native or introduced), as well as their interaction, on the probability of flowering in 2005 (Proc Genmod, SAS). Median threshold size is determined as the size at which the probability of flowering is 0.5 (Wesselingh et al. 1997). We used Type III Likelihood Ratio tests to assess significance of the model factors in this analysis.

In all analyses of variance, statistical significance of fixed factors was tested using F-tests based on Type III sums of squares, where the error term was calculated from the appropriate combination of random effects. The appropriate denominator degrees of freedom for models with random factors were determined by Satterthwaite's approximation (Satterthwaite 1946). Plant volume and fecundity were natural log transformed in all analyses to meet model assumptions of equal variance.

Results

Field common gardens in the native and introduced ranges

All of the plants in the common gardens on both continents either flowered in their second year or did not survive to flower at all. Survival in the German garden was high (90%) for both native and introduced populations. In the Montana garden, survival of plants from native European populations (77%) was significantly higher than that of plants from introduced North American populations (54%; $\chi_1^2 = 4.93$, $P = 0.026$).

Populations exhibited substantial plasticity in size and fecundity between gardens. In general, plants grown in Germany were much larger and produced more seeds than plants grown in Montana (Fig. 1d, e; Table 3). Introduced and native populations responded differently to the respective growing conditions across gardens, indicated by the significant range of origin by garden interaction (Table 3). Specifically, the magnitude of plasticity of introduced populations was greater, on average, than that of native populations. Introduced populations were, on average, 4659% larger and produced 2344% more seeds in the German garden compared to the Montana garden. This response was higher than that of native populations, which were, on average, 2912% larger and produced 1246% more seeds in the German garden compared to the Montana garden. Although, plants from populations in both ranges

flowered earlier in the German garden, on average, we observed no differences in the magnitude of plasticity in flowering phenology between populations from the native and introduced ranges (Fig. 1f; Table 3).

Across gardens, populations exhibited differences in the magnitude of phenotypic plasticity for all traits measured (population(range) × garden was significant; Table 3). However, this significant population differentiation in plasticity was driven by significant differences among native populations. When plants from the native and introduced ranges were analyzed separately, we found no significant differences among introduced populations (population × garden interaction was not significant; plant volume: $F_{9,129} = 1.33$, $P = 0.23$; fecundity: $F_{9,124} = 1.41$, $P = 0.19$; day of first flowering: $F_{9,127} = 0.57$, $P = 0.82$). In contrast, plasticity in all traits was significantly different among native populations (significant population × garden interaction; plant volume: $F_{9,153} = 3.29$, $P = 0.001$; fecundity: $F_{9,147} = 2.08$, $P = 0.035$; day of first flowering: $F_{9,166} = 2.66$, $P = 0.007$).

In the German garden, plants from introduced populations were larger (Fig. 1d; Tukey posthoc test: $P < 0.001$) and produced more seeds (Fig. 1e; Tukey post hoc test: $P = 0.006$) than those from native populations. In contrast, in the Montana garden, plants from native populations produced slightly more seeds on average than those from introduced populations, although these differences were not statistically significant (Fig. 1e; Tukey post hoc test: $P = 0.24$). In contrast to plant size and fecundity, the day of first flowering showed a very different pattern both within and between gardens (Fig. 1c, f). Plants from both ranges flowered earlier in the German garden and on average, native populations flowered earlier than introduced populations in both gardens (Fig. 1f; Table 3).

Table 3 Results from ANOVA testing for plasticity of plant volume, fecundity and date of first flowering between common gardens in Germany and Montana

	F	df	P
Plant volume			
Garden	1271.96	1, 18.8	**<0.001**
Range of origin	26.46	1, 20.2	**<0.001**
Range × garden	5.96	1, 18.8	**0.025**
Population (range)	0.36	18, 18.0	0.98
Population (range) × garden	2.53	18, 279	**<0.001**
Fecundity (total seed production)			
Garden	609.85	1, 19.0	**<0.001**
Range of origin	0.76	1, 20.8	0.39
Range × garden	7.70	1, 19.0	**0.012**
Population (range)	0.38	18, 18.0	0.98
Population (range) × garden	1.79	18, 271	**0.026**
Date of first flowering (Julian day)			
Garden	146.83	1, 19.0	**<0.001**
Range of origin	3.90	1, 18.1	0.064
Range × garden	0.02	1, 19.0	0.89
Population (range)	6.81	18, 18.0	**<0.001**
Population (range) × garden	1.86	18, 271	**0.019**

Significant values ($P < 0.05$) highlighted in bold

Nutrient addition experiment

Populations from both ranges responded positively to nutrient additions, with plants in the high nutrient treatment attaining significantly larger size in the first year and higher fecundity in the second year (Fig. 2a, b; Table 4). In all treatments, populations from the introduced range were, on average, larger or produced more seeds. However, the strength of this plastic response was not higher for

Fig. 1 Norms of reaction for plant volume (**a**, **d**), fecundity (**b**, **e**), and day of first flowering (**c**, **f**). Both plant volume and fecundity are natural log transformed. **a–c** Show plasticity across gardens, with each line representing mean trait values for individuals from different native (*solid lines*) and introduced (*dashed lines*) populations. **d–f** Show averages of population means for each range (native or introduced), with *error bars* representing one SE of the mean; when error bars are not visible, they are obscured by the points
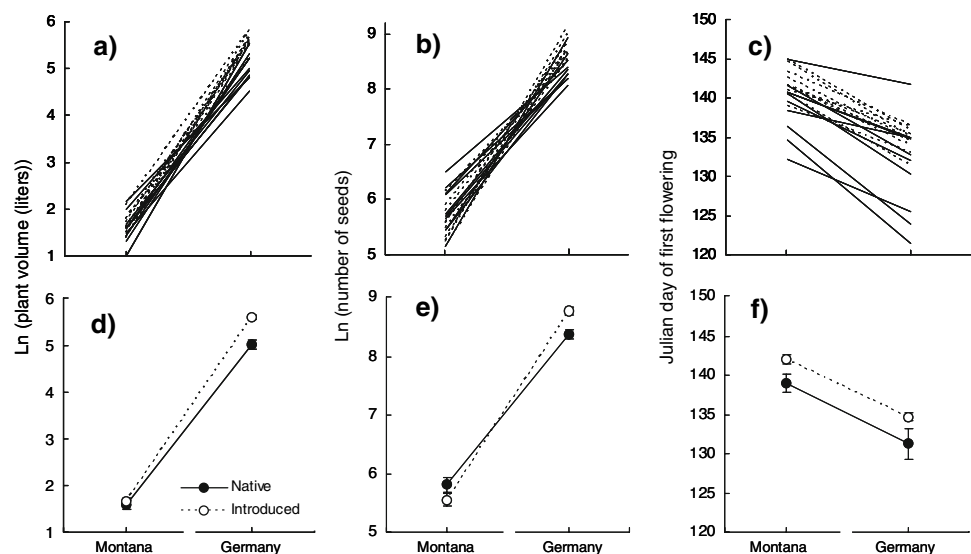
introduced populations as indicated by the non-significant interaction between nutrient treatment and range of origin (Table 4). Day of first flowering did not change between nutrient treatments, but occurred marginally significantly earlier in native populations (Fig. 2c; Table 4). We observed significant variation among populations for day of first flowering and plant size, but not for fecundity (Table 4).

Although we expected that individuals in the low nutrient treatment might not reach the threshold flowering size, the majority of plants that survived to their second year flowered (97%). Vegetative size was a strong predictor of the probability of flowering ($\chi^2_1 = 52.67$, $P < 0.001$), but we found no significant difference in median threshold flowering size between plants from native and introduced populations in the pot experiment (range: $\chi^2_1 = 2.65$, $P = 0.10$; range × size: $\chi^2_1 = 0.95$, $P = 0.33$). We were unable to detect differences in threshold flowering size in the main common garden experiments, because all plants either flowered in their second year or did not survive.

Discussion

Our study demonstrates the importance of using more than one environment to examine evolutionary changes in invasive plants. The substantial differences in size and fecundity between plants grown in the Montana and German common gardens (Fig. 1d, e) demonstrate that *C. officinale* from both native and introduced populations can respond dramatically to different growing conditions. The fact that introduced populations of *C. officinale* were larger and more fecund, but only in experiments in the native range (Germany), highlights the potential pitfalls of interpreting data collected in only one common environment. Had we conducted experiments only in Germany, we might have concluded that introduced populations had evolved to be larger, and used that as a possible explanation for the success of this invasive plant. However, this was not true in the common garden in the introduced range (Montana), where we observed no significant differences between continent of origin for plant size or fecundity and in fact,

Table 4 Results from an ANOVA testing for plasticity in plant volume, fecundity and date of first flowering from nutrient addition (pot) experiment

	F	df	P
Plant volume			
Range of origin	17.67	1, 18.0	**<0.001**
Nutrient level	162.97	2, 36.2	**<0.001**
Range × nutrient level	0.35	2, 36.2	0.70
Population (range)	3.62	18, 36.1	**<0.001**
Population (range) × nutrient	0.58	36, 296	0.98
Fecundity (total seed production)			
Range of origin	5.23	1, 18.4	**0.034**
Nutrient level	41.68	2, 32.8	**<0.001**
Range × nutrient level	1.43	2, 32.8	0.25
Population (range)	1.27	18, 37.1	0.26
Population (range) × nutrient	0.95	36, 237	0.56
Date of first flowering (Julian day)			
Range of origin	3.74	1, 18.1	0.069
Nutrient level	0.71	2, 37.7	0.50
Range × nutrient level	0.33	2, 37.7	0.72
Population (range)	18.54	18, 36.6	**<0.001**
Population (range) × nutrient	1.09	36, 252	0.34

Significant values ($P < 0.05$) highlighted in bold

plants from populations from the native range were slightly more fecund. Taken together, our contrasting results across gardens do not support the EICA hypothesis, as we found no consistent evidence that introduced populations were significantly larger or more fecund than native populations. However, our results dramatically illustrate that the genetically based differences between native and introduced populations that the EICA hypothesis predicts (Blossey and Nötzold 1995) may not be revealed unless experiments are conducted in more than one location. Additionally, while plasticity may be an important contributor to exotic plant success, its role cannot be determined without growing plants in multiple gardens or habitats.

Even with multiple gardens, a challenge in testing the EICA hypothesis is that common gardens are often assumed to be representative of conditions in the range in which they are located. Yet, no one site can adequately

Fig. 2 Norms of reaction for plant volume (**a**), fecundity (**b**) and day of first flowering (**c**) from the nutrient experiment. Both plant volume and fecundity are natural log transformed. Panels show averages (±1 SEM) of population means for each range (native or introduced) at low, medium or high nutrient levels
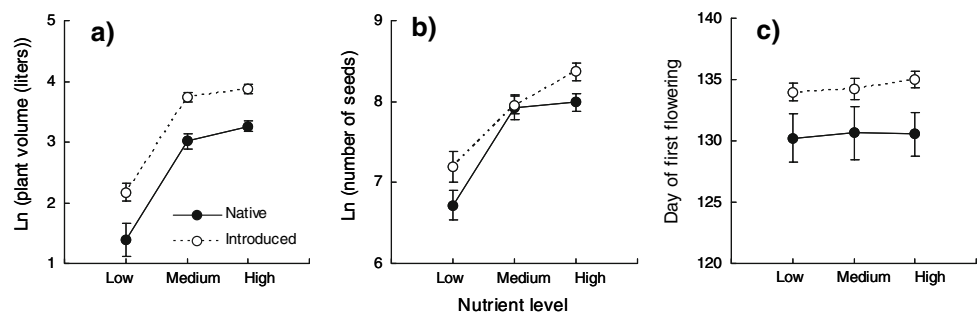
represent conditions across either the entire native or introduced range. In our case, although the gardens represented differences in climate between the Rocky Mountains and Europe, edaphic conditions did not necessarily reflect differences between ranges. For example, plants growing in the German garden were much larger than those occurring in natural populations in either the native or introduced range (J. Williams, unpublished data). Although we found that soil nitrogen content was higher in the Montana garden, we measured the total pool size of N rather than plant-available nitrogen. In Germany, higher nutrient availability at the garden site and a milder growing season, with less extreme summer and winter temperatures and higher summer rainfall, likely explain the absolute size differences between gardens. Differences in size and fecundity were less pronounced in the nutrient addition experiment, with both increasing only slightly between the medium and high fertilizer treatments (Fig. 2a, b). These results suggest that factors other than nutrients, potentially size of pots, limited growth and seed production.

Phenotypic plasticity across gardens for size and fecundity was generally higher among introduced populations compared to native populations. Although one might interpret these results as evidence for the evolution of increased plasticity within the introduced range, a more likely explanation may be that founder effects played a strong role in creating the differences we observed. We base this interpretation on three lines of evidence. First, we found no genetically based phenotypic differentiation in plasticity of size and fecundity among introduced populations and yet significant among population variation in plasticity for size and fecundity among native populations. Second, given the wide variety of habitats where introduced populations occur, in the absence of founder effects it is unlikely that all introduced populations would evolve in a unidirectional way to produce relatively low among-population variation in plasticity. Finally, recent genetic analyses involving more populations than used in our common garden experiments indicate that both allelic diversity and average heterozygosity are lower among individuals from introduced populations compared to native populations (J. Williams, unpublished data). This suggests that introduced populations represent only a subset of diversity found within the native range. It may be that founding genotypes in the native range originated from a portion of Europe where plasticity is particularly high.

The fact that we found substantial plasticity in size and fecundity raises the question of whether such plasticity is adaptive. One possibility is that the large differences in plasticity for traits strongly associated with fitness (fecundity and size) reflects much lower levels of plasticity in underlying physiological traits that directly influence fitness. If physiological traits are more canalized, it could

result in reductions in fitness in sites where the environment differs from optimal, since physiological traits would lack the ability to plastically compensate for suboptimal conditions. In a similar reciprocal common garden study involving the invasive plant, *Hypericum perforatum*, Maron et al. (2007) found significantly greater plasticity in size and fecundity than in physiological traits such as water use efficiency and leaf nitrogen.

Unlike our results for size and fecundity, plasticity in date of first flowering showed a very different pattern. Plants from both ranges flowered earlier when growing in Germany than in Montana (Fig. 1f). However, no plasticity in date of first flowering was observed for plants from either range grown at different nutrient levels within the same garden (Fig. 2c). These contrasting results suggest that climatic conditions and the length of the growing season are more important in controlling when plants flower than nutrient availability. Other studies have found similar patterns for date of first flowering in common gardens at different latitudes (Clausen et al. 1940; Griffith and Watson 2006; Jonas and Geber 1999; Lacey 1988). Similar to the fitness related traits we measured, only populations from the native range displayed a significant amount of variation among populations (Fig. 1c). This narrow range of variation and lack of differentiation in introduced compared to native populations offers further support for the presence of a founder effect in introduced populations of *C. officinale*.

Life history theory predicts that threshold flowering size should increase when the probability of pre-reproductive mortality decreases (Roff 1992; Wesselingh et al. 1997), as might be the case with introduced plants that escape their specialist enemies. However, we found no evidence that an evolutionary change in threshold flowering size has occurred in *C. officinale*. In the nutrient experiment, the vast majority of plants attained threshold size in their first year and were able to flower in the second. In addition, even if plants in the introduced range in North America wait to attain a larger size before flowering, we might not detect this in a common environment with high levels of resources. Instead, when growing conditions are favorable, as in our experiment, plants appear to be able to acquire enough resources to surpass a minimum threshold size.

In conclusion, we found no consistent advantage in size or fecundity of *C. officinale* for introduced populations across gardens, thus offering no support for the EICA hypothesis. However, we did find plasticity for size, fecundity and date of first flowering, with plants able to respond to more favorable environments. This ability to take advantage of favorable growing conditions has long been attributed to weedy species, particularly those that occur in disturbed habitats (Baker 1965). For phenotypic plasticity to explain the increased success of *C. officinale*

where it is introduced, we would need to observe higher levels of plasticity in introduced populations for traits that confer a fitness advantage (Richards et al. 2006). Our measurements of average population level plasticity do not fully address the possibility of adaptive plasticity. Rather, our results point to the potential for founder effects to be important among introduced populations. This hypothesis is supported by the lack of differentiation among introduced populations and the narrower range of variation in traits among introduced versus native populations, together with recently analyzed genetic data (J. Williams, unpublished data). Future studies of the role of evolution in invasive plants could benefit by explicit consideration of the role of genetic by environmental interactions in affecting the results of common garden experiments.

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