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Research article

Analyzing reaction norm variation in the field vs. greenhouse: Comparing studies of plasticity and its adaptive value in two species of *Erodium*

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ABSTRACT

Despite a wide range of experiments characterizing patterns of selection on phenotypic plasticity in controlled environments there has been virtually no research assessing the extent to which these results reflect selection on plasticity expressed in natural populations. To test how well the patterns observed in controlled experiments match the patterns in field populations, we present two case studies in which we characterized the fitness consequences of plasticity both under controlled lath house conditions and in the field. We quantified selection on plasticity in response to soil nutrient variation in two annual plant species, *Erodium cicutarium* and *Erodium brachycarpum*. For both species, families collected from the same source populations were used in both field and lath house experiments. We ask whether the qualitative results obtained from field and controlled environment experiments are equivalent. In two cases we observed selection on the expression of plasticity by *E. brachycarpum* in the field while controlled environment experiments indicated that plasticity was selectively neutral. In three other cases we observed differences in the pattern of plasticity expressed in the controlled environment experiment relative to the field resulting in conflicting results regarding the form of trait expression favored by selection. Based on these results, we argue that the extent to which results from controlled environments can be accurately extrapolated to naturally occurring populations depends on whether treatments imposed in a controlled environment accurately mimic environmental variation in the field and induce plasticity in traits of interest. Ideally any controlled environment experiment characterizing plasticity would be paired with field survey data of environmental and phenotypic variation within naturally occurring populations.

Introduction

Throughout the process of translating initial observations of a natural system into hypotheses, and subsequently designing experiments to test hypotheses, researchers must balance several significant trade-offs. In studies focused on characterizing variation in trait expression and fitness the choice between conducting an experiment in an organism's natural habitat ("field" experiments), or in a controlled environment, will determine the feasible number of replicates and the precision with which phenotypic traits and fitness can be measured (Diamond, 1986). The majority of experiments characterizing the fitness consequences of phenotypic plasticity (both adaptive and maladaptive) have been conducted in controlled environmental settings, for example greenhouses, growth chambers or labs (except see Dudley and Schmitt, 1996; Winn, 1999; Donohue et al., 2001; Galen et al., 2004; Huber et al., 2004; Galloway and Etterson, 2007; Baythavong and Stanton, 2010; Baythavong, 2011). In many cases the motivation for characterizing selection on plasticity in the lab or greenhouse is clear; it is logistically very challenging to measure phenotypic plasticity in the field. Measuring the fitness consequences of genetic variation and phenotypic plasticity in trait expression requires several steps (Schlichting, 1986; Via et al., 1995; Bischoff and Muller-Scharer, 2010). First, replicate genotypes (ex. full sibling families or clones) should be generated in a common garden generation to reduce the expression of maternal environmental effects. Second, individuals from each genotype must be exposed to either to continuous variation within naturally occurring populations, or discrete environments in either the lab or field. Third, a proxy for lifetime fitness (viable seed number in annual plants) and multiple phenotypic traits, ideally spanning several developmental stages, should be measured on each experimental individual.

As in a traditional genotypic selection analysis (Lande and Arnold, 1983), the statistical power available to test for selection on phenotypic plasticity is primarily determined by the number of genotypes included in an experiment (Schlichting, 1986; Via et al., 1995). Because genotypes should be replicated to provide

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a stabilized estimate of family mean trait expression and fitness within each environment, the sample sizes necessary to measure selection on phenotypic plasticity can be daunting, especially when variation in more than one environmental factor is manipulated. In some study systems it may not be possible to achieve the sample sizes required to detect a statistically significant result in the field, where emergence rates are lower and mortality is higher, or due to restrictions on field collections of original seed stocks (ex. rare species). Field experiments are also vulnerable to unanticipated natural variation not associated with the hypotheses being tested (ex. errant mudslides, previously undocumented granivorous insects, extreme droughts), and it is often difficult to measure traits accurately in the field.

A central theme in theoretical explanations for range expansions in invasive species and anticipated range shifts in response to climate change is the balance between the expression of genetic variation and phenotypic plasticity in heterogeneous landscapes (Bradshaw, 1965; Booy et al., 2000; Agrawal, 2001; Richards et al., 2006; Crispo, 2008; Schlichting, 2008; Nicotra et al., 2010). Consistent differences in the qualitative conclusions from controlled environment and field experiments characterizing plasticity could bias our understanding of the role of plastic trait expression in establishing range limits in invasive species and the ability of species to respond to environmental change (Chevin et al., 2010). Despite these potential limitations, however, to our knowledge no one has explicitly compared differences in the qualitative conclusions about plasticity drawn from controlled environment and field experiments.

We present analyses of data from four experiments conducted in the field and lath house to measure the fitness consequences of plasticity expressed by two invasive annual plant species, *Erodium brachycarpum* and *Erodium cicutarium*. Our controlled environment experiments assayed phenotypic plasticity and fitness expressed in response to discrete fertilization treatments. We chose to manipulate nutrient availability in the lath house because results from previous field surveys demonstrated it is highly heterogeneous in the grassland where both species were collected (Baythavong, 2011), and because edaphic variation is generally important in determining the distribution of co-occurring annual plant species (Brady et al., 2005; Murren et al., 2006; Lau et al., 2008). We analyze patterns of selection on plasticity using a standard genotypic selection analysis. The goal of these analyses is to provide an example of typical methods used to characterize phenotypic plasticity and selection in a controlled environment. We compare the results from the lath house experiments to field experiments on the same species characterizing plasticity in the same traits in response to continuous natural variation in macro- and micro-nutrient availability. Because the seeds used for both species were originally collected from the same source populations in the same year, these represent parallel pairs of field and controlled environment experiments that allow us to compare those two approaches directly.

Specifically, we present analyses of all four data sets to address the following questions:

1. Is the magnitude and direction of plasticity expressed in the greenhouse similar to plasticity expressed in response to continuous variation in the field?
2. How does selection act on mean trait expression and phenotypic plasticity expressed in response to low and high nutrient treatments in the greenhouse?
3. How does selection act on the expression of phenotypic plasticity in response to continuous variation in nutrient availability in the field?
4. Are the results describing patterns of selection and phenotypic plasticity qualitatively similar when the same species is planted into controlled environment and field conditions?

Materials and methods

Study system: E. cicutarium and E. brachycarpum in Northern California

E. brachycarpum and *E. cicutarium* are annual plants of Mediterranean origin that have invaded a diverse array of habitats throughout California, including serpentine soils (Baythavong et al., 2009), ruderal fields, and non-serpentine grasslands (Rice, 1985; Stamp, 1989). Mensing and Byrne (1998) estimate that *E. cicutarium* became established in California in the 1700s. Both species are self-compatible and readily produce self-fertilized seeds in the field and greenhouse (Baythavong and Stanton, 2010). Populations of both species are broadly distributed across a heterogeneous mosaic of serpentine and non-serpentine soils within the University of California's (UC) Donald and Sylvia McLaughlin reserve in Northern California (38.8246° N, 122.3438° W). As a result of the broad distribution of *E. brachycarpum* and *E. cicutarium* across serpentine and non-serpentine grasslands, adjacent populations of both species experience striking differences in macro- and micro-nutrient availability. We used an existing extensive soil dataset available at the reserve to identify six populations of each species which grow in a wide range of macro- and micro-nutrient availability on serpentine and non-serpentine soils. Additional information about the criteria used to select populations is available in Baythavong and Stanton (2010).

Experimental design

Field collections and common garden generation

Equivalent field collection and common garden generation greenhouse methods were used for both *E. cicutarium* and *E. brachycarpum* and are described in detail in Baythavong and Stanton (2010). At the end of the common garden generation all plants were allowed to produce self-fertilized seeds (180 families per species) which were collected and stored in cool dry conditions and used in subsequent lath house and field experiments (see Table 1).

Lath house experiments

In March 2005 thirteen full sibling families of *E. brachycarpum* and *E. cicutarium* from six populations (4 serpentine and 2 non-serpentine) at the UC McLaughlin reserve were randomly selected (78 families per species) from the 180 generated in the common garden generation for each species. Four seeds per full sibling family were planted and germinated following the same protocol used in the previous common garden generation. Following emergence one seedling per full sibling family was randomly assigned to a high nutrient treatment and a second seedling was assigned to a low nutrient treatment in lath houses on the UC Davis campus. Seedlings in the high nutrient treatment were sub irrigated three times per week with fertilizer water as in the previous common garden generation, and seedlings in the low nutrient treatment were sub irrigated three times per week with reverse osmosis water (Jacobs and Lesmeister, 2012). The fertilizer water available for use in the lath houses at UC Davis is supplemented with calcium nitrate, magnesium sulfate, and Grow More brand injectible fertilizer which contains a 4:18:38 mix of NPK as well as added Fe, Zn, Mn, Mg, Cu, Mb, B and S. To facilitate a comparison of macro- and micro-nutrient availability between lath house and field experiments soil from eight conetainers supplied with fertilizer water three times per week for the entire lifespan of *E. cicutarium* was

pooled and analyzed by A and L Agricultural labs for organic matter (OM), expected nitrogen release (ENR), HCO_3P, K, Mg, Ca, Na, and SO_4S availability. An existing analysis of the UC mix potting soil provides an estimate of nutrient availability encountered in the nutrient stress treatment at the beginning of the experiment; this provides an estimate of the highest level of fertility experienced by plants in the nutrient stress treatment since plants would have depleted these nutrients over the course of the experiment (Table A1).

Several traits were measured on every surviving individual throughout the experiment. Rosette leaves were counted approximately two weeks after emergence (early leaf number). Leaf longevity was measured on all experimental plants by placing a miniscule drop of Liquid Paper™ white out on the tip of the youngest developing leaf approximately 3 weeks after emergence. Leaves were recorded as senesced after less than 10% of the surface area was green. Additionally, the surface area of one randomly selected fully expanded leaf per plant was measured 6 weeks after emergence. First flower date was surveyed every other day and recorded for each surviving individual. The number of viable seeds produced by each individual was used as a proxy for lifetime fitness.

Analyses of plasticity in early leaf number, leaf area, and first flower date were conducted for *E. brachycarpum*, and analyses of plasticity in early leaf number, leaf longevity, and first flower date were conducted for *E. cicutarium*. These traits were selected based on five criteria: (1) they were measured both in the field and in the lath house for each species, (2) they characterize patterns of morphological and phenological variation at equivalent life stages in both species, (3) they were feasible to measure on a large number of individuals in the lath house and field, (4) they represent trait variation at several stages throughout the lifespan of *Erodium* (Via et al., 1995), and (5) they have been shown to be associated with adaptation to low nutrient availability in other annual plant species (Chapin III, 1980). Because each full sibling family was represented by only one individual in each treatment, families with at least one individual missing were not included in analyses of plasticity. After mortality, 76 full sibling families of *E. cicutarium* and 63 families of *E. brachycarpum* were available for subsequent analyses.

E. brachycarpum field experiment

In fall 2009 we planted replicate families of *E. brachycarpum* produced in the original common garden generation in a ruderal field in the Putah Creek Riparian Reserve located near the UC Davis campus (38.528315° N, 121.785181° W). Thirty experimental blocks were arranged in clusters of five in the field within an area approximately 25 m by 50 m. The locations of clusters were selected prior to planting to span the range of above ground competition present at the site (3 high competition, 3 low competition) based on assessments of the standing biomass present from the previous growing season.

Each seed was physically scarified to break dormancy and attached to a wooden toothpick using a miniscule amount of water soluble glue before planting. Seven individuals from the thirty randomly selected grandmaternal families (representing 5 of the 6 original source sites) were planted into thirty experimental blocks in the field (210 seeds per block, 6300 seeds total). Seeds from each grandmaternal family were randomly assigned locations within each experimental block and planted at 4 cm intervals (blocks measured 56 cm by 60 cm) in October 2009.

Reserve managers at the Putah Creek Riparian reserve introduce cattle to the field in which the experiment was planted 2–3 times per year. Each time cattle are introduced they are allowed to graze the vegetation down to a height of approximately 15 cm to control the growth and reproduction of invasive plant species. After planting, we enclosed the experiment in an electrified fence to protect the blocks from destruction by trampling. Each time the surrounding field was grazed; all vegetation within each block (and within a 1 m radius of each block) was clipped with hand shears to approximately the same height as the surrounding grazed vegetation to approximate the grazing treatment used by reserve managers. As a result, clipping the blocks enabled us to mimic conditions experienced throughout the distribution of *E. brachycarpum* in California, which frequently occurs in high abundance in grazed pastures (Rice, 1990). The tops of larger *Erodium* plants were often clipped by this simulated grazing treatment, but these responded by sending up additional stems and flower stalks, so that clipping did not produce a noticeable change in the rank order of individual plant size. This compensatory response to clipping is consistent with previous work that showed that in a grassland population, clipping reduced growth and fitness of *E. cicutarium* by only about 10% compared to unclipped plants (Kimball and Schiffman, 2003).

Several traits were measured on each surviving individual including the number of rosette leaves (early leaf number) after most seedlings had emerged (December 2009). The length and width of the largest rosette leaf available on each surviving individual was measured using digital calipers in January 2010. Leaf area and width were multiplied to provide an approximation for leaf area. Beginning in mid-February plants were surveyed on two week intervals for flowering. First flower dates spanned a range of 71 days (March 1st–May 15th), and plants were in flower for many days after the last plant initiated flowering. Lifetime fitness was estimated by counting the number of viable seeds produced by each surviving individual. Viable seed number can be estimated accurately in *E. brachycarpum* by collecting all fruits after a plant has dispersed its seeds because each fruit contains five ovules and inviable seeds remain attached to the fruit, while viable seeds are dispersed (Rice, 1985).

In December 2009 soil cores were collected from two randomly selected corners of each experimental block and dried to a constant weight at 70 °C. Soil chemistry was measured on one core per block. Soil chemistry analyses, conducted by A and L Agricultural labs, included expected nitrogen release (ENR), P (weak bray), HCO_3P, K, Mg, Ca, Na, pH, H, cation exchange capacity (CEC), and SO_4S.

E. cicutarium field experiment

A detailed description of the field experiment is available in Baythavong and Stanton (2010). We provide a brief summary here for reference. Beginning in October 2006 one full sibling seed from 30 maternal families per original source population (180 families total) was randomly assigned a location within each experimental block in the field. Seeds were planted into five blocks per original collection site in the field according to a full reciprocal transplant design at the UC McLaughlin reserve (5 blocks per site, 6 sites, 180 seeds per block, 5400 seeds total). Emergence timing, cotyledon width, early leaf number, leaf longevity, leaf turnover, and first flower date were measured. After approximately half the plants in each block had initiated bolting soil samples were taken from all four corners of each block. Soil chemistry, including ENR, CEC, OM, P (weak bray), HCO_3P, pH, Na, K, Ca, Mg, Fe, Zn, Cu, and Mn, was analyzed on each soil sample by A and L Agricultural labs.

Data analysis

Characterize average phenotypic plasticity in morphological and phenological traits expressed in response to variation in nutrient availability

Lath house experiments. Mixed model analyses of variance (ANOVA) were used to test for phenotypic plasticity and variation in trait expression in the lath house for each species separately (the MIXED procedure; SAS). The ANOVA model used to analyze individual

traits measured in the lath house included the main fixed effects of source soil type (serpentine or non-serpentine), source site (nested within source soil type), and nutrient treatment (high or low nutrients), as well as the pair wise interactions between source soil type and treatment and source site and treatment. Family (nested within source soil type and source site) and rack (nested within treatment) were included as random effects. When a significant effect of source soil type, source site, treatment, or any of the pair wise interactions was detected a-posteriori Tukey tests were used to compare means. Levene's tests revealed significant heteroscedasticity in all variables expressed by both species, except leaf longevity (*E. cicutarium*), among full sibling families. As a result, all ANOVAs were weighted by the reciprocal of the variance within each family for each trait to reduce heteroscedasticity (Stanton and Thiede, 2005).

Field experiments. A principal components analysis (PCA) was used to characterize patterns of variation in all macro- and micronutrients analyzed on soil samples taken from each block in the field (the PRINCOMP procedure; SAS). We used regressions to characterize the average pattern of plasticity in each trait expressed across all maternal full sibling (*E. cicutarium*) or grandmaternal (*E. brachycarpum*) families in response to principal component axes (the REG procedure; SAS). The value for each trait was included as the dependent variable and the value of each environmental variable, and the squared value of that environmental variable, were included as predictor variables (3 traits, 2 axes of environmental variation, 6 models total per species). A significant relationship between a trait and an environmental variable indicates an average linear plastic response across all experimental families, and a significant effect of the squared environmental variable indicates an average curvilinear plastic response across all experimental families. Statistical significance of analyses conducted for *E. brachycarpum* was assessed after Bonferroni correction for multiple tests (three regressions per environmental variable) at the $P = 0.0167$ level. A more stringent correction for multiple tests was used ($P = 0.007143$) to analyze the experiment presented in Baythavong and Stanton (2010) because more traits were considered (7 total).

Selection on mean trait expression and phenotypic plasticity in response to variation in nutrient availability

Lath house experiments. Multiple linear regressions (MLR) were used to assess selection on family mean trait values and on plasticity. To the extent that the selfed maternal families used in the lath house represent genotypes, our approach can be viewed as a genotypic selection analysis with one representative per family in each treatment (Lande and Arnold, 1983). For each species the average fitness of each full sibling family across high and low nutrient treatments was regressed against the standardized mean trait values for each full sibling family expressed across both treatments and the standardized difference in trait expression for each family between high and low fertilizer treatments ($X_{high} - X_{low}$) (the REG procedure; SAS). All traits measured were included together in one multiple linear regression analysis per species to account for potential correlated effects among traits on fitness. A signficant effect of mean trait values on fitness indicates that selection favors consistant trait values expressed across both environments, while an effect of the difference in trait values from high to low nutrient treatments indicates selection on the expression of phenotypic plasticity (Stinchcombe et al., 2004, Fig. A1). However, to determine the extent to which significant selection on the difference in trait expression between treatments (and on reaction norms in the field experiments, below) reflects selection for adaptive plasticity, or selection against maladaptive plasticity, requires careful examination of the relationship between fitness and the form of plasticity expressed by each full sibling family (Baythavong and Stanton, 2010, Fig. A1).

E. brachycarpum and E. cicutarium field experiments. The same statistical analyses were used in Baythavong and Stanton (2010) to characterize selection on mean trait expression and plasticity in traits expressed by full sibling families of *E. cicutarium* in the field. Although we do not present a re-analysis of this dataset here, we provided brief descriptions of the statistical analyses and results characterizing average patterns of plasticity and selection on continuous reaction norms in the field for reference.

Individual simple linear regressions between the trait values expressed by *E. brachycarpum* and the value of the environmental variable measured in each block (PC 1 and 2) were conducted by grandmaternal familiy for each trait by environmental axis combination. For traits which exhibited an average curvilinear response to a given environmental factor (PC 1 and 2) a second set of simple linear regressions was conducted which included both the value of that environmental factor and the squared value of that environmental factor. The linear slope, curvilinear slope (when applicable), and intercept for each trait by environmental axis were standardized across the entire experiment.

The relationship between grandmaternal family mean fitness (across the entire experiment) and the standarized intercept, linear slope, and curvilinear slope (where appropriate) of reaction norms was assessed using multiple linear regressions (the REG procedure; SAS) to account for the effects of inter-trait correlations on fitness. This resulted in two multiple linear regression analyses, one for each of the two edaphic principal components considered. As a result, overall model significance was assessed after Bonferroni correction for multiple tests at the $P = 0.025$ level.

Results

Patterns of mean trait expression and plasticity in response to variation in nutrient availability

E. brachycarpum lath house experiment

All three traits measured in the lath house expressed significant plasticity in response to high and low nutrient availability treatments (Table 2a). Early leaf number was significantly greater in high nutrient treatments (lsmean = 6.5053, std error = 0.1413) than in low nutrient treatments (lsmean = 4.4897, std error = 0.1751). Plants flowered significantly later in low nutrient treatments (lsmean flowering date = May 22nd, std error = 1.6385) than in high nutrient treatments (lsmean flowering date = April 6th, std error = 1.3042). Leaf area was also consistantly higher in high nutrient treatments (lsmean = 2.8994, std error = 0.2014) than low nutrient treatments (lsmean = 1.1857, std error = 0.2614), although the magnitude of this average plastic response varied among source populations (Table 2a).

E. cicutarium lath house experiment

On average full sibling families of *E. cicutarium* expressed plasticity in early leaf number and first flower date in response to nutrient treatments in the lath house (Table 2b). Early leaf number was significantly greater in high nutrient treatments (lsmean = 8.0954, std error = 0.1346) than in low nutrient treatments (lsmean = 5.5939, std error = 0.1648), and the magntiude (not direction) of this plastic response varied among source soil types and source sites (Table 2b). *E. cicutarium* families flowered significantly earlier in high nutrient treatments (lsmean flowering date = April 3rd, std error = 0.7284) than in low nutrient treatments (lsmean flowering date = April 16th, std error = 0.8608). We observed that the magnitude (not direction) of plasticity in first

Table 1
Comparison of the experimental designs used to characterize plasticity and fitness in *E. brachycarpum* (EB) and *E. cicutarium* (EC) in the lath house (LH) and field.

Species	Experiment	Unit of replication	# of families	Environmental factors manipulated	# of environments	Analysis to test for plasticity
EB	LH	Full sibling families	63	Fertilizer/no fertilizer	2	ANOVA
	Field	Grandmaternal families	30	Micro- and macro-nutrients	30	Regression
EC	LH	Full sibling families	76	Fertilizer/no fertilizer	2	ANOVA
	Field	Full sibling families	180	Heavy metals, micro-, and macro-nutrients	27	Regression

Table 2
Univariate mixed model ANOVAs of early leaf number, leaf area, leaf longevity, and first flower date expressed in the lath house by *E. brachycarpum* (a) and *E. cicutarium* (b). In all models treatment, source soil type, source site, and all interactions were analyzed as fixed effects.

Effect	(a) *E. brachycarpum*						(b) *E. cicutarium*					
	Early leaf #		Leaf area		First flower date		Early leaf #		Leaf longevity		First flower date	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	93.00	<0.0001	28.20	0.0005	531.65	<0.0001	158.35	<0.0001	0.13	0.7272	151.22	<0.0001
Source soil type	0.01	0.9065	4.39	0.0413	0.37	0.5454	0.28	0.5965	0.45	0.5068	3.57	0.0631
Source site (source soil type)	0.03	0.8920	0.67	0.6141	1.04	0.3974	11.51	<0.0001	1.29	0.2833	4.71	0.0021
Source soil type × treatment	1.90	0.1755	0.03	0.8635	2.66	0.1095	4.65	0.0350	0.06	0.8061	16.28	0.0002
Source site (source soil type) × treatment	0.56	0.6907	7.54	0.0001	2.04	0.1042	4.71	0.0022	0.55	0.7024	5.84	0.0005

flower date changed between source soil types and source popoulations (Table 2b).

E. brachycarpum field experiment

Principal components analysis of soil chemistry data revealed two orthogonal principal components that explained approximately 68% of the total variation (Table 3). Principal component 1 describes an axis of variation in micronutrient availability, including K, Mg, Ca, Na, CEC, and SO_4S. Principal component 2 describes an axis of variation in macronutrient availability including OM, ENR, P, and HCO_3P.

Across all grandmaternal families, we detected significant plasticity in all traits in response to variation in micronutrient availability (PC 1). Early leaf number increased in response to increasing micronutrient availability (PC 1, linear slope = 0.1611, curvilinear slope = 0.0285, $P < 0.0001$). Leaf area also increased in response to increasing micronutrient availability (PC 1, linear slope = 0.2178, $P < 0.0001$). In contrast, first flower date decreased in response to micronutrient availability (PC 1, linear slope = −1.4106, $P = 0.0002$). On average, individuals in sites with low levels of micronutrients flowered later than individuals in sites with higher levels of micronutrients.

Early leaf number and leaf area also responded to differences in macronutrient availability (PC 2) among blocks. On average, individuals produced more rosette leaves in sites with lower levels of macronutrient availability, although leaf numbers were maximized at intermediate values (PC 2, linear slope = −0.3521, curvilinear slope = −0.0716, $P < 0.0001$). Similarly, leaf area increased in response to diminishing macronutrient availability and maximum leaf areas were produced in sites with intermediate values (PC 2, linear slope = −0.3037, curvilinear slope = −0.0600, $P < 0.0001$).

Table 3
Principal components analysis of all soil chemistry data collected at 30 experimental blocks in the *E. brachycarpum* field experiment. Loadings of all soil chemistry variables onto the first two principal components, eigen values, and the proportion of variance explained by each PC are shown.

	Prin1	Prin2
Eigen value	5.7469	2.4466
Prop. variance explained	47.89%	20.39%
OM	0.12803	0.52700
ENR	0.12026	0.52830
P	0.21203	0.36584
HCO_3P	0.24771	0.34165
K	0.36602	0.04598
Mg	0.38001	−0.16201
Ca	0.37069	−0.09981
Na	0.29197	−0.17963
pH	−0.0476	0.20292
H	0.21919	−0.21244
CEC	0.39893	−0.14959
SO_4S	0.39029	−0.11085

E. cicutarium field experiment

The principal components analyses presented in Baythavong and Stanton (2010) describe variation in four multivariate axes of edaphic variation. Generally, micronutrients load negatively onto PCs 1–3 and macronutrients load positively onto PCs 1–3. The opposite pattern was reported for PC 4, on which macronutrients load negatively and micronutrients load positively (Table A2).

As presented in Baythavong and Stanton (2010) *E. cicutarium* expressed significant plasticity in early leaf number, leaf longevity, and first flower date in response to all four edaphic principal components axes (Table A3). Although the first four principal components axes did not represent distinct sets of macro- and micro-nutrients, early leaf number generally increased in response to increasing micronutrient availability and decreased in response to increasing macronutrient availability (significant negative reaction norm slopes in response to PCs 1–3). Leaf longevity was greatest in sites with low levels of macronutrients, and lowest in sites with high levels of macronutrients (significant negative linear relationship with PCs 1 and 2). Families flowered later in sites with greater macronutrient availability and flowered earlier in sites with greater micronutrient availability (significant negative linear relationship with PCs 1 and 2).

Patterns of selection on mean trait expression and phenotypic plasticity in response to variation in micro- and macro-nutrient availability

E. brachycarpum lath house experiment

Overall we detected significant selection on trait expression in response to lath house treatments (df = 6, $F = 2.36$, $P = 0.0454$). Selection favored increased plasticity in first flower date in response to lath house treatments ($\beta = -5.9906$, $P = 0.0196$, Fig. 1g). Variation in mean first flower date ($\beta = -5.1137$, $P = 0.0705$), early leaf number ($\beta = 0.2053$, $P = 0.9270$), and leaf area ($\beta = 1.2584$, $P = 0.7148$) was selectively neutral.

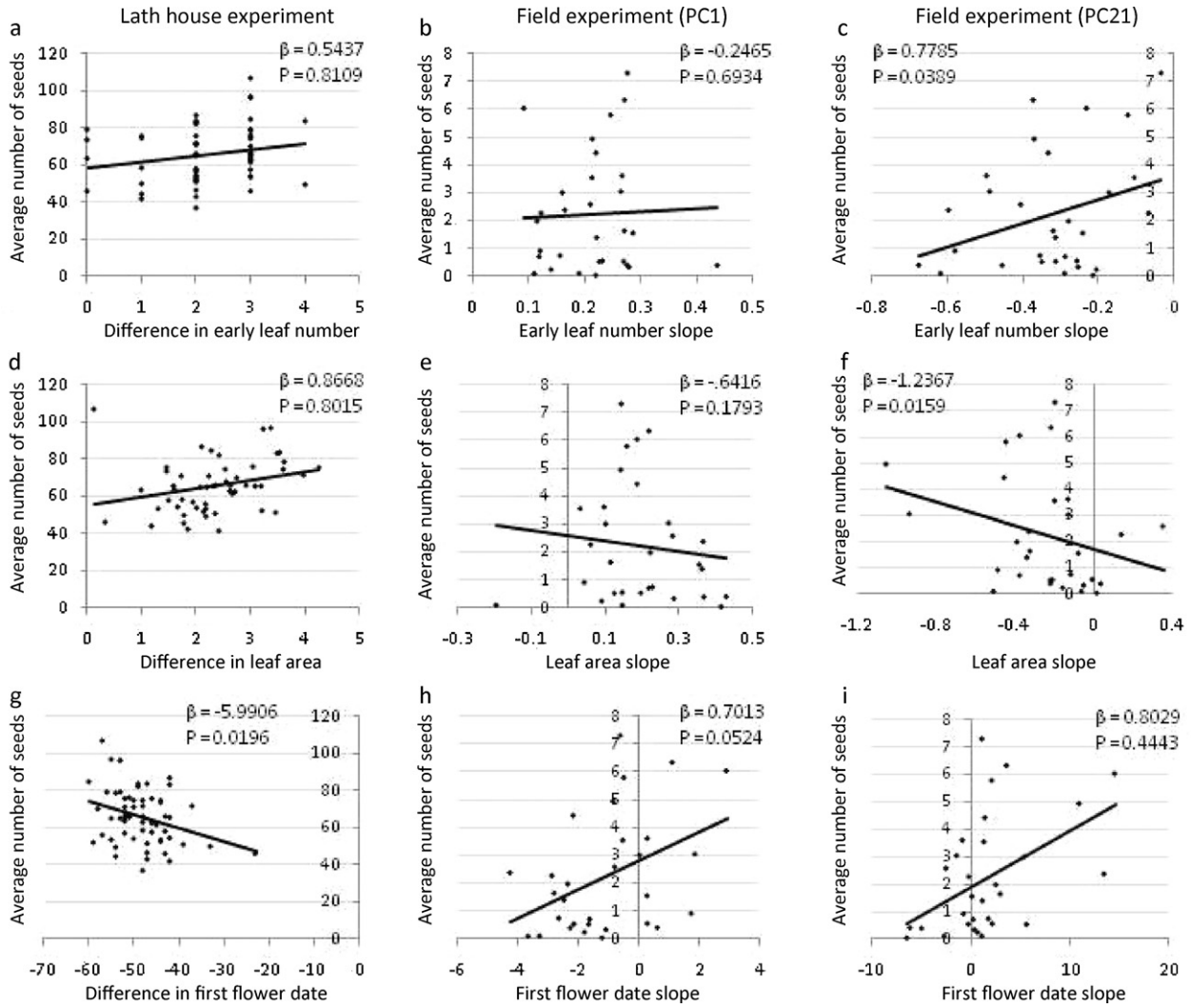

Fig. 1. Relationship between average fitness of each *E. brachycarpum* experimental family and the linear reaction norm slope of each family in response to lath house fertilizer treatments (a, d, g) and continuous variation in micronutrient (b, e, h) and macronutrient (c, f, i) availability in the field. Partial regression coefficients (β) and *P*-values for individual factors within three multiple linear regression are shown. Univariate lines of best fit between average fitness and reaction norm slope included.

E. cicutarium lath house experiment

We detected significant selection on both average trait expression and plasticity expressed by *E. cicutarium* in response to fertilizer treatments in the lath house (df = 6, $F = 9.28$, $P < 0.0001$). Selection favored reduced mean leaf longevity ($\beta = -6.7113$, $P = 0.0069$) and earlier mean first flower date ($\beta = -11.5243$, $P = 0.0004$) across both lath house treatments. Additionally, families that produced more leaves in high nutrient treatments relative to low nutrient treatments ($\beta = 7.8808$, $P = 0.0017$, Fig. 2a) and expressed greater plasticity in first flower date ($\beta = -8.9422$, $P = 0.0048$, Fig. 2e) had the highest average fitness. We found a non-significant trend suggesting selection favoring families which decreased leaf longevity in response to increasing macronutrient availability (Fig. 2c; $\beta = -4.5470$, $P = 0.0682$).

E. brachycarpum field experiment

Selection on mean trait expression (reaction norm intercepts) and plasticity in trait expression (reaction norm slopes) in reponse to variation in micro- (PC 1, df = 7, $F = 3.25$, $P = 0.0158$) and macro- (PC 2, df = 8, $F = 4.14$, $P = 0.0042$) nutrient availability was apparent in the field. Grandmaternal families which produced larger rosette leaves, on average, had higher fitness ($\beta = 1.0543$, $P = 0.0137$). Additionally, families that flowered later in sites with high micronutrient availabilty, and earlier in microsites with low micronutrient availability tended to have the highest overall fitness (PC 1, $\beta = 0.7013$, $P = 0.0524$, Fig. 1h).

Although we did not detect significant selection on mean early leaf number (reaction norm intercept, $\beta = 0.6463$, $P = 0.0922$), families which expressed diminished plasticity (zero reaction norm slope) in leaf number had the highest mean fitness (PC 2, $\beta = 0.7785$, $P = 0.0389$, Fig. 1c) in response to macronutrient variation. In contrast, families which expressed stronger negative linear reaction norm slopes in leaf area ($\beta = -1.2367$, $P = 0.0159$) in response variation in macronutrient availability had the highest overall fitness (Fig. 1f). We also observed a marginally significant trend for selection favoring families which expressed more dramatic negative curvature in leaf area reaction norms in response to variation in macronutrient availability ($\beta = -0.9790$, $P = 0.0509$).

E. cicutarium field experiment

Results of selection analyses are presented in Baythavong and Stanton (2010). Although not statistically significant, there was a consistent trend favoring increased mean early leaf number (postive selection on reaction norm mid point) across all axes of edaphic

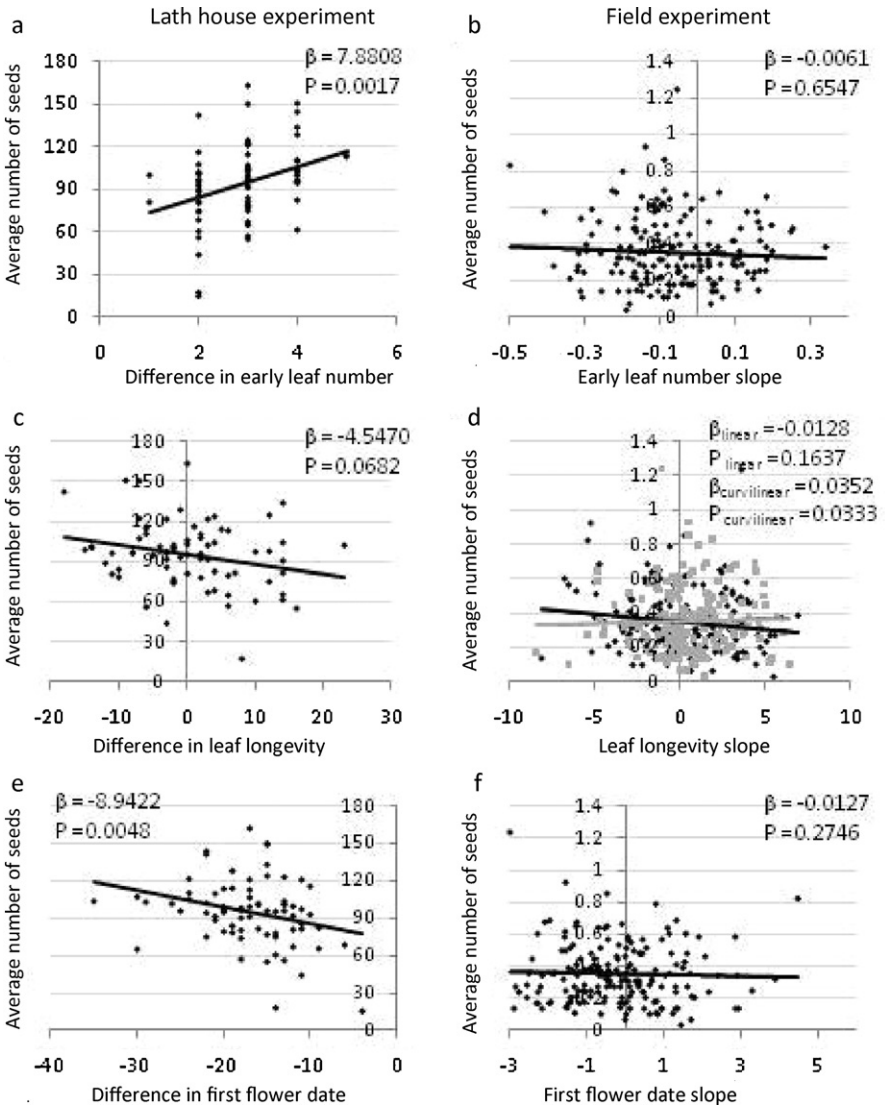

Fig. 2. Relationship between average fitness of each *E. cicutarium* experimental family and linear reaction norm slope in response to lath house fertilizer treatments (a, c, e) and continuous variation in macronutrient (b, d, f) availability (PC 2) in the field. For simplicity only results of selection analyses on plasticity expressed in response to one axis of edaphic variation in the field (PC 2) are shown. Betas and *P*-values for individual factors within separate multiple linear regression models used to test for a significant relationship between family mean fitness and reaction norm midpoint and slope are shown. Univariate lines of best fit between average fitness and reaction norm slope included.

variation (Table A2). Baythavong and Stanton (2010) also demonstrated consistant statistically significant selection for earlier mean first flower dates, but no significant selection on plasticity in first flower date (Table A2). In contrast, selection analyses revealed significant positive selection on the curvilinear slope of continuous leaf longevity reaction norms expressed in response to an axis of variation in macro- (OM, ENR, and HCO_3P) and micro- (Mn and Cu) nutrient variation (Table A2). Families of *E. cicutarium* which increased leaf longevity in sites with high levels of micronutrients, and decreased leaf longevity in sites with high levels of macronutrients had higher fitness than less plastic families (Table A4).

Discussion

The adaptive value of phenotypic plasticity is overwhelmingly measured in controlled environment experiments (for review see Bradshaw, 1965; Schlichting, 1986; Bradshaw and Hardwick, 1989; Ghalambor et al., 2007). To our knowledge no studies have explicitly compared the results and qualitative conclusions from selection analyses on the same traits, and their plastic responses, in both controlled environment and field conditions in families of the same species. Our results suggest that there are some consistent qualitative differences between conclusions drawn from controlled environment and field experiments characterizing selection on phenotypic plasticity expressed by *E. cicutarium* and *E. brachycarpum* (Table 4).

Comparison of the direction and magnitude of plasticity expressed in controlled environment and field conditions

A qualitative comparison of results from the lath house and field experiments revealed that lath house fertilizer treatments elicited average plastic responses similar to only a subset of the continuous multivariate environmental variation present in the field. For *E. brachycarpum*, the average direction of plasticity expressed in all traits measured in the lath house clearly resembled the patterns of plasticity expressed in the field in response to micronutrient variation (PC 1), but not macronutrient variation (PC 2, Fig. 1).

Table 4
Summary of the results from analyses of all four lath house (LH) and field experiments. The direction of plasticity in response to increasing nutrient availability in the field and lath house (a), the sign of significant selection differentials on average trait expression (b), plasticity in trait expression (c) and the adaptive value of plasticity in each trait is shown (d). Marginally insignificant results are indicated with an asterisk.

Experiment	Species	Trait	(a) Response to nutrient availability		(b) Selection on trait mean	(c) Selection on plasticity		(d) Adaptive plasticity	
			Micro	Macro		Micro	Macro	Micro	Macro
LH	*E. brachycarpum*	Early leaf #	+		0	0			
		Leaf area	+		0	0			
		First flower date	–		0	–			Y
Field		Early leaf #	+	–	0	0	+		N
		Leaf area	+	–	+	0	–		Y
		First flower date	–	0	0	+*	0	Y	
LH	*E. cicutarium*	Early leaf #		+	+	+			Y
		Leaf longevity		0	–	–*			
		First flower date		–	–	–			Y
Field		Early leaf #	+	–	+*	0	0		
		Leaf longevity	+	–	0	+	–	Y	Y
		First flower date	–	+	–*	0	0		

Additionally, while we observed both positive and negative reaction norm slopes in response to environmental variation in the field (Fig. 1f, h, and i), only one form of plastic response for each trait (either positive or negative reaction norms) was observed in the lath house (Fig. 1d and g). For example, in the lath house, families of both *E. brachycarpum* and *E. cicutarium* consistently increased leaf area and flowered earlier in response to increasing nutrient availability. In contrast, when planted in the field, some families expressed the opposite response and decreased leaf area and flowered later in response to increasing nutrient availability. Baythavong and Stanton (2010) demonstrated increased leaf longevity in sites with both extremely low and extremely high availability, but reduced leaf longevity in intermediate microsites, in the field (Fig. 2d). Because lath house treatments only provided a discrete comparison between two extreme environments with high and low micronutrient availability, without a range of intermediate environments, the strong curvilinear plastic response in leaf longevity observed in the field was not detected in the lath house (Table 2b).

Comparisons of nutrient availability observed in field experiments and lath house treatments demonstrate that lath house treatments generally fell within the range observed in the field, with only two exceptions (Table A1). High levels of Na and S availability observed in the lath house are likely the result of factors endemic to all greenhouse experiments, application of pesticides and fungicides (ex. foliar SO_4 to control powdery mildew) and accumulation of salts in small pots over time. Because there is no consistent trend for a narrower range of nutrient availabilities between treatments in the lath house relative to the field experiments it is unlikely that this accounts for the consistent differences in the expression of plasticity observed in the lath house and field. Additionally, because families used in all experiments were randomly sampled from the same common garden seed stocks, and in some cases the same families were planted in to both experimental contexts (*E. cicutarium*), it is unlikely that sampling biases affect our results.

Instead, this strong difference in the pattern of plasticity expressed in the lath house and field in both species is the result of three important differences between lath house treatments and field environments. First, in the field micro- and macro-nutrients either varied independently (*E. brachycarpum*), or were negatively correlated (*E. cicutarium*). In the lath house the fertilizer contained both micro- and macro-nutrients, so micro- and macro-nutrient availability were tightly positively correlated. Second, in the *E. brachycarpum* field experiment macronutrient variation was positively correlated with above ground interspecific competition ($\rho = 0.29$). Third, when continuous environmental variation in the field is reduced into discrete treatments in the lab it is not possible to accurately characterize plasticity in traits that express strongly curvilinear reaction norms (Kingsolver and Gomulkiewicz, 2003).

Comparison of the adaptive value of plasticity in controlled environment and field experiments

Analyses of selection on plasticity revealed substantial differences in the qualitative conclusions about the adaptive value of plasticity expressed by both species between lath house and field contexts. Two examples of selection on plasticity expressed by *E. brachycarpum* in the field were not detected in the lath house: (1) selection favored *E. brachycarpum* families which expressed the most dramatic decreases in leaf area in response to macronutrient variation (Fig. 1f) and (2) families with the highest average fitness expressed diminished plasticity in early leaf number in response to variation in macronutrient availability (PC 2, Fig. 1c). In the lath house, plasticity in these traits was selectively neutral (Fig. 1a and d). Because changes in leaf number and leaf area in response to nutrient treatments mimicked the average positive plastic responses to micronutrient variation in the field (Fig. 1b and e) the fitness consequences of plasticity in early leaf number and leaf area expressed in response to macronutrient variation were only apparent in the more complex field environment. Similarly, our experiments characterizing selection on traits expressed by *E. cicutarium* also resulted in contrasting qualitative conclusions about the adaptive value of plasticity. Selection favored increased plasticity in early leaf number and first flower date in response to lath house treatments (Fig. 2a and e). However, plasticity in the same traits in response to all axes of soil nutrient variation was selectively neutral when measured in the field (Table A4).

In one case controlled environment and field experiments resulted in contrasting results regarding the *form* of plastic trait expression favored by selection. On average, families of *E. brachycarpum* flowered later in environments with low levels of micronutrients and earlier in environments with high levels of micronutrients when measured in both the lath house and field. Selection analyses in the lath house indicated that this average plastic response was adaptive (Fig. 1g). In contrast, when planted in the field, the few *E. brachycarpum* families which expressed the opposite plastic response and flowered earlier in low micronutrient sites tended to have higher fitness (marginally significant effect, Fig. 1h).

The results from our greenhouse experiments characterizing the adaptive value of phenotypic plasticity in response to edaphic variation are qualitatively different from field observations.

Although we detected adaptive plasticity in response to greenhouse treatments, the same responses to macronutrient variation were neutral, or maladaptive, in the field. Additionally, two examples of adaptive plasticity in first flower date (*E. brachycarpum*) and leaf longevity (*E. cicutarium*) expressed in response to micronutrient variation in the field were not detected in the greenhouse. Adaptive phenotypic plasticity is hypothesized to be important in enabling rapid range expansions in invasive species, or in enabling species to shift their range in response to climate change (Sexton et al., 2002; Richards et al., 2006; Crispo, 2008; Schlichting, 2008; Chevin et al., 2010; Nicotra et al., 2010). The results of our controlled environment experiments discount the role of plasticity in response to micronutrient variation in facilitating the success of *Erodium* species in California.

Conclusions

A qualitative comparison of the results from these four experiments demonstrates several weaknesses in using controlled environment experiments to measure selection on phenotypic plasticity. Plasticity expressed by both species in response to the nutrient availability treatments imposed in the lath house only resembled patterns of plasticity expressed in response to a subset of the continuous multivariate environmental variation present in the field. Overall, these results indicate that measuring selection on plasticity is more effective in the field where responses to multiple co-varying environmental factors can be assessed simultaneously. The same qualitative trend has been reported in other study systems. Anderson et al. (2010) also compared measurements of phenotypic plasticity in response to a complex multivariate environmental gradient in the field, and in response to water availability treatments in the greenhouse. Although they detected plasticity in response to water availability in the field, genotypes expressed reduced plasticity in foliar traits in the greenhouse.

In some cases it is may not be possible to study selection on plasticity in the field due to limited funding, ethical restrictions on releasing genotypes in the field, or limited source material available for rare or endangered species. If, for a given study system, it is more appropriate to measure fitness and plasticity in a controlled environment it is important to combine the results with field surveys. Although field survey data could substantially strengthen conclusions drawn from controlled environment experiments, very few studies characterizing phenotypic plasticity in the lab are conducted in combination with comprehensive field surveys of phenotypic and environmental variation (except see Anderson et al., 2010). Before designing greenhouse or lab experiments researchers should combine surveys of phenotypic and environmental variation in the field within and among naturally occurring populations. Results from a priori field surveys should inform several aspects of experimental design and analysis including: (1) identifying axes of environmental variation which co-vary most strongly with variation in the traits of interest, (2) establishing treatment levels to be imposed in the lab based on the range of environmental variation measured in the field, and (3) establishing a point of comparison to verify that patterns of adaptive plasticity observed in the lab resemble phenotypic variation expressed in the field. A combined analysis of controlled environment experiments with field data to characterize adaptive plasticity should enhance studies of both native and invasive plant species. Adaptive plasticity is hypothesized to play an important role in facilitating invasive species establishment and spread, as well as persistence of native species in heterogeneous habitats and in response to climate change (Agrawal, 2001; Sexton et al., 2002).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ppees.2012.04.002.

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