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Young Jin Chun
Iowa State University

Michael L. Collyer
Iowa State University

Kirk A. Moloney
Iowa State University, kmoloney@iastate.edu

John D. Nason
Iowa State University, jnason@iastate.edu

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Keywords

common garden, genotype X environment (G X E) interactions, invasive species, Lythrum salicaria, multivariate analysis, native vs. invasive, phenotypic plasticity, purple loosestrife, reaction norm

Disciplines

Ecology and Evolutionary Biology | Other Ecology and Evolutionary Biology | Statistical Methodology | Terrestrial and Aquatic Ecology

PHENOTYPIC PLASTICITY OF NATIVE VS. INVASIVE PURPLE LOOSESTRIFE: A TWO-STATE MULTIVARIATE APPROACH

Young Jin Chun,[1] Michael L. Collyer,[1,2] Kirk A. Moloney,[1,3] and John D. Nason[1]

[1]*Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, Iowa 50011 USA*
[2]*Department of Statistics, Iowa State University, Ames, Iowa 50011 USA*

Abstract. The differences in phenotypic plasticity between invasive (North American) and native (German) provenances of the invasive plant *Lythrum salicaria* (purple loosestrife) were examined using a multivariate reaction norm approach testing two important attributes of reaction norms described by multivariate vectors of phenotypic change: the magnitude and direction of mean trait differences between environments. Data were collected for six life history traits from native and invasive plants using a split-plot design with experimentally manipulated water and nutrient levels. We found significant differences between native and invasive plants in multivariate phenotypic plasticity for comparisons between low and high water treatments within low nutrient levels, between low and high nutrient levels within high water treatments, and for comparisons that included both a water and nutrient level change. The significant genotype × environment (G × E) effects support the argument that invasiveness of purple loosestrife is closely associated with the interaction of high levels of soil nutrient and flooding water regime. Our results indicate that native and invasive plants take different strategies for growth and reproduction; native plants flowered earlier and allocated more to flower production, while invasive plants exhibited an extended period of vegetative growth before flowering to increase height and allocation to clonal reproduction, which may contribute to increased fitness and invasiveness in subsequent years.

Key words: common garden; genotype × environment (G × E) interactions; invasive species; Lythrum salicaria; *multivariate analysis; native vs. invasive; phenotypic plasticity; purple loosestrife; reaction norm.*

INTRODUCTION

Invasive species are a source of global environmental change, harming native species and communities, altering ecosystem processes in invaded areas, and causing enormous economic damage (Vitousek et al. 1996, Simberloff 2000). Although considerable research has focused on invasive species (Mooney and Hobbs 2000), little consensus exists about the underlying strategies of species that allow them to become successful invaders (cf. Williamson 1999, Kolar and Lodge 2001, Sakai et al. 2001, Mack et al. 2002). Nonetheless, phenotypic plasticity has historically been seen as a potentially important mechanism for colonization success in environmentally diverse areas and may play a role in the process of invasion (Marshall and Jain 1968, Rice and Mack 1991, Williams et al. 1995, Kaufman and Smouse 2001, Maron et al. 2004). Phenotypic plasticity is simply a phenotypic response to environmental variation; however, adaptive plasticity is a response that ultimately enhances function and maximizes fitness through highly specific developmental, physiological, and reproductive adjustments to local environmental conditions (Sultan 2003). Therefore,

adaptive plasticity is a more appropriate concept to explore in examining successful invasion than is phenotypic plasticity. Adaptive plasticity may contribute to invasive ability by allowing the acclimation of invasive genotypes to diverse environments, as well as buffering existing genetic variation from selection, thereby reducing the necessity for local adaptation (Schlichting 1986, Sultan 2003). Therefore, adaptive plasticity may lead to phenotypic homeostasis (tolerance) in fitness, which can be important in the process of successful invasion (Rejmánek 2000, Alpert and Simms 2002).

Adaptive plasticity has been detected repeatedly among invasive plant species. As an example, in one study Sultan and Bazzaz (1993*a*) found that *Polygonum persicaria* grown under flooding produced dense mats of finely branched superficial and adventitious roots at the soil surface, such that growth was maintained by avoiding the deleterious effects of oxygen deficits. In a second study (Sultan and Bazzaz 1993*b*), they found that at different nutrient levels, *P. persicaria* maintained the same photosynthetic surface area relative to total biomass and the same leaf nitrogen concentration. Since adaptive plasticity is often expressed through interactions among multiple traits rather than through a single trait, such characteristics in a phenotypic response argue for the analysis of multiple traits contributing to fitness

in studying the relationship between adaptive plasticity and invasiveness.

In this paper, we use purple loosestrife (*Lythrum salicaria* L.) as a model system to investigate the relationship between adaptive plasticity and invasiveness. This was done in an experimental study designed to contrast the multivariate plastic response of plants derived from native and invasive source populations to varying environmental conditions. Purple loosestrife is an herbaceous perennial plant and a well-known, aggressive invader of wetlands in North America (Thompson et al. 1987). Introduced to North America in the early 1800s (Stuckey 1980), it has spread rapidly throughout the continent and changed the basic structure of most wetlands that it has invaded (Blossey et al. 2001). New recruits into a population are primarily from seed (Stevens et al. 1997b), but as plants increase in size they can produce multiple shoots originating from a single root crown. The breeding system of purple loosestrife has been of particular interest since it is a tristylous species, exhibiting three distinct flower types that require outcrossing for successful fertilization (Agren and Ericson 1996). Seed production is massive, with an individual plant producing ~900 seed capsules with 120 seeds per capsule (Shamsi and Whitehead 1974a). Seeds are light (0.5–0.6 mg), but wind dispersal is often limited to 10 m (Thompson et al. 1987). The most likely mode of spread is by wetland wildlife through ingestion or external adherence of seed with subsequent deposition (Thompson et al. 1987).

There is some evidence that purple loosestrife is more vigorous in North America than in Europe. Invasive populations in North America appear to be characterized generally by higher densities (Edwards et al. 1998, Bastlová-Hanzélyová 2001), taller and larger plants (Blossey and Nötzold 1995, Bastlová-Hanzélyová 2001, Bastlová and Květ 2002), and more fertile shoots (Edwards et al. 1998) than populations in native European habitat. Although there has been some work examining purple loosestrife in the context of its response to varying environmental conditions (Shamsi and Whitehead 1974a, b, 1977a, b, Mal et al. 1997a, Stevens et al. 1997a, Edwards et al. 1998, Bastlová et al. 2004), there has not been a study adopting a multivariate reaction norm approach to relate its invasiveness to the evolution of phenotypic plasticity.

While classical studies in evolutionary ecology have tended to focus on variation in single characters, there is increasing interest in covariation among characters and the impact this has on phenotypic evolution (Waldmann and Anderson 2000). The idea that an organism's phenotype cannot be easily characterized by a single trait is illustrated in the concepts of genetic correlation (Falconer and MacKay 1996) and phenotypic integration (Wagner and Altenberg 1996, Pigliucci 2003). An organism's phenotype may be treated as a set of multivariate variables, whereby the covariation of traits that are important in phenotypic evolution is considered

in the analysis of phenotypic differentiation. It is particularly interesting to study the relationship between phenotypic integration and environmental variation (i.e., how the environment can alter the patterns of phenotypic correlation among traits, and how this response may differ between native and nonnative populations). The contrast between the adaptive advantages of integrated responses (Schlichting 1986, Schlichting and Pigliucci 1998) vs. their nonadaptive roles as indicators of constraints (Gould 1984) is fundamental to our understanding of phenotypic evolution (Pigliucci and Schlichting 1995, 1998). Even though methods have been developed to quantify natural selection acting on multivariate phenotypes (Lande and Arnold 1983), there has been a dearth of applications of these methods to measurements taken in different environments.

To address this issue, we adopt a recently developed two-state multivariate vector approach (Collyer and Adams 2007) for comparing phenotypic responses among different source populations. Phenotypic change within a population is depicted through vectors of mean trait differences between contrasting environments (e.g., multivariate reaction norm analysis). The two-state approach contrasts the multivariate response between populations in two ways. First, a comparison of the magnitude of vectors can be used to determine if invasive populations exhibit greater multivariate plasticity than native ones. A second analysis quantifies directional differences between vectors in multivariate trait space to determine if native and invasive populations exhibit different patterns of trait covariation. Such directional differences may provide further insight into the evolution of life history strategies associated with invasiveness. Using this multivariate approach, we address two basic questions concerning phenotypic plasticity and invasiveness in purple loosestrife. (1) When plants are exposed to a change in water and nutrient levels, do native and invasive populations exhibit different patterns in the magnitude and direction of their multivariate reaction norms? (2) Are the differences in the magnitude and direction of multivariate reaction norms between native and invasive populations consistent with a change in adaptive plasticity of invasive populations, which may produce greater fitness? To address these questions, we conducted a common garden study in which three populations each from the native (Europe) and invasive (North America) provenances were subject to experimentally manipulated water and nutrient levels. Differences between provenances in the amount and direction of multivariate phenotypic change were then determined for a set of six vegetative and reproductive characters.

UNIVARIATE ANALYSIS OF REACTION NORMS

The reaction norm is a function of phenotypic values across environments that can be described for individuals or for populations (Via et al. 1995, Pigliucci 2001). The traditional analysis of reaction norms involves a univar-
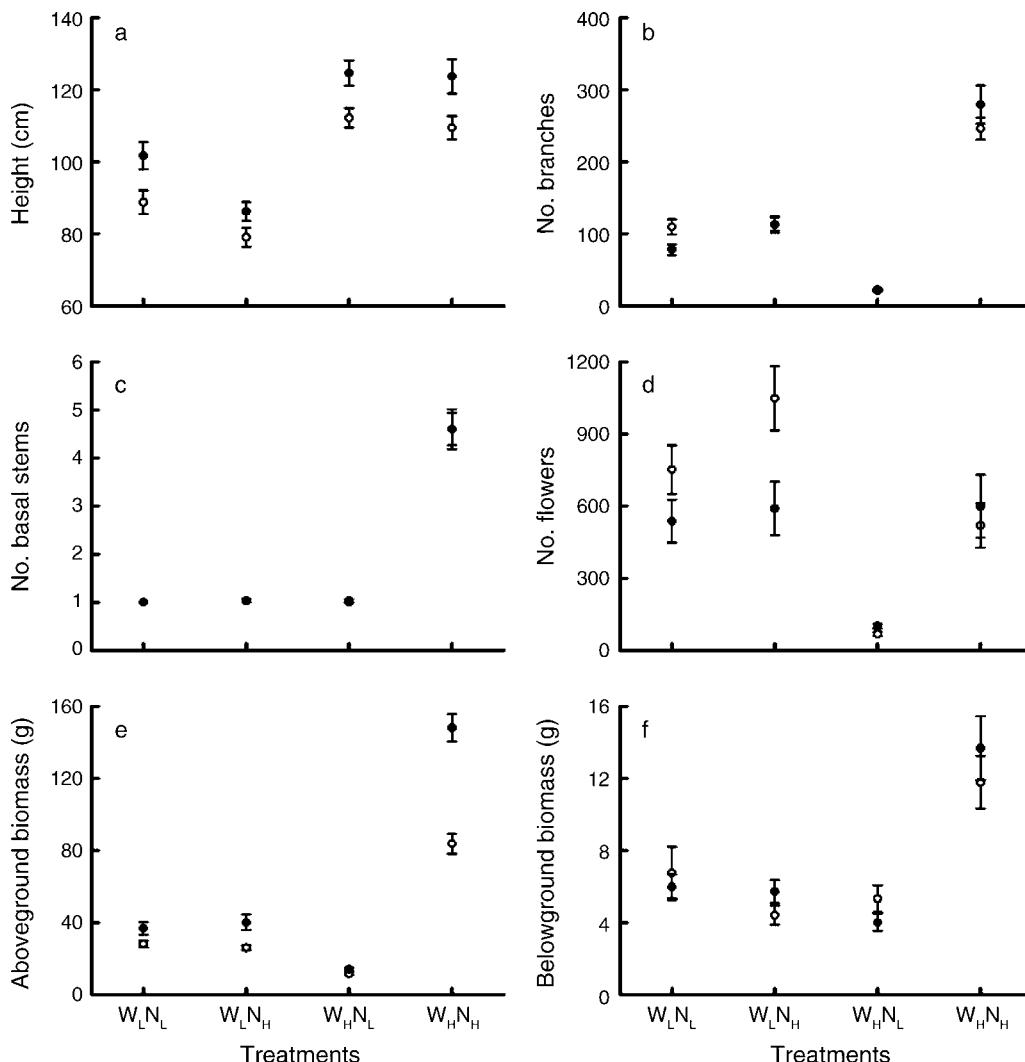
FIG. 1. Least-square means (±SE) of six trait values for plants from native (open circles) and invasive (solid circles) provenances. Environmental treatments: $W_L N_L$, low water, low nutrient; $W_L N_H$, low water, high nutrient; $W_H N_L$, high water, low nutrient; $W_H N_H$, high water, high nutrient.

iate approach, examining reaction norms for each trait separately. (The same univariate approach has also been applied to synthetic metrics, such as PCA scores, that are often used to reduce the dimensionality of multivariate data sets when there is a high degree of collinearity among traits.) To assess the effect of G (genetic variation), E (environment), and G × E interactions on phenotypic responses, ANOVA is conducted for each trait. Significant G × E effects indicate significant genetic variation for phenotypic plasticity.

To infer a biological explanation for genetic variation in phenotypic plasticity, one can examine G × E means plotted trait by trait. For example, four G × E means for six different traits are plotted for purple loosestrife in Fig. 1. Although such plots easily indicate why a significant G × E interaction would be observed for a single trait in an ANOVA (e.g., a change in the rank of phenotypic values

between two environments, as in Fig. 1b, d, e, f), it is practically impossible to gain an understanding of whether there is genetic variation in phenotypic plasticity in terms of trait covariation (i.e., plasticity of phenotypic integration). This is especially problematic for traits that are not independent. For univariate data, the reaction norm simply describes the magnitude of difference in trait values between environments. For multivariate data, reaction norms are more complex because of potential negative or positive covariance among traits. Therefore, an analytical approach is needed that accounts for the covariation of multiple traits.

APPLICATION OF TWO-STATE MULTIVARIATE ANALYSIS

G, E, and G × E effects can be statistically analyzed with multivariate analysis of variance (MANOVA) to determine if there are significant genetic differences in
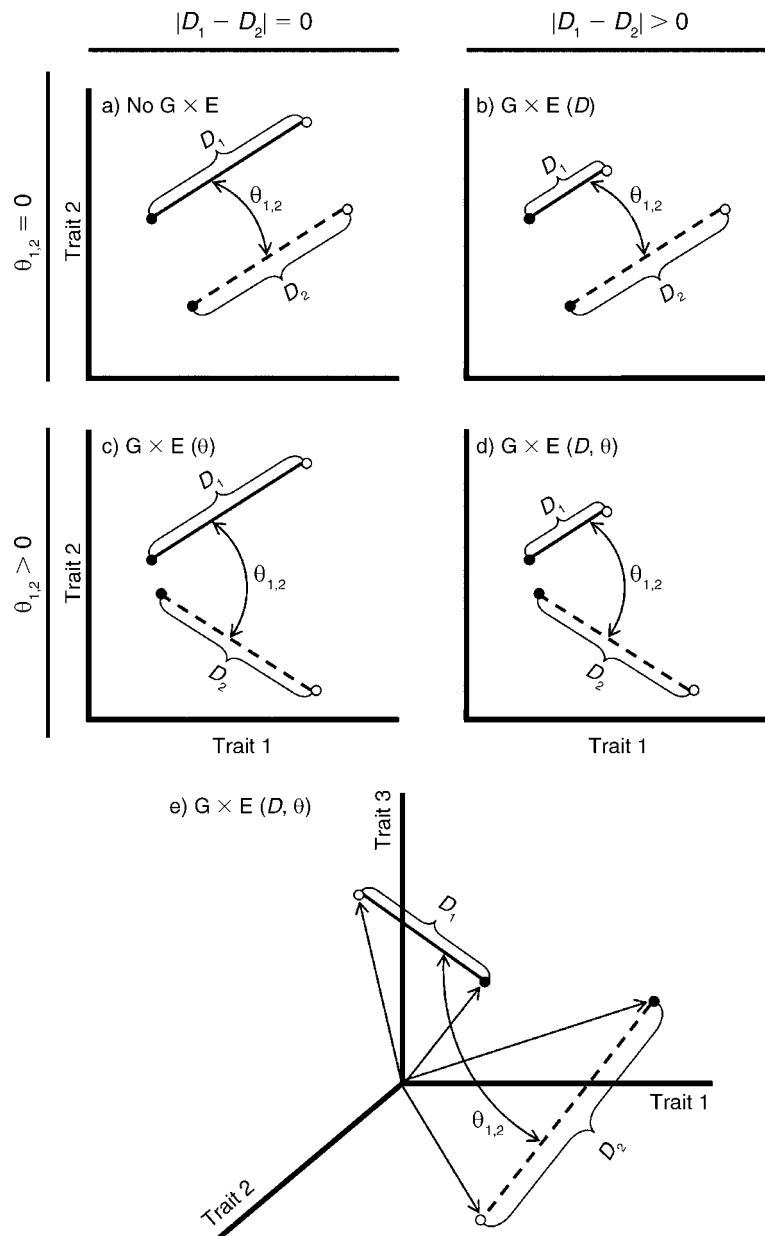
FIG. 2. Hypothetical reaction norms for two genotypes (G) or populations (indexed 1 and 2) and two environments (E, open and solid circles) in bivariate (a, b, c, d) and multivariate (e) trait space. Solid and dashed lines denote reaction norm vectors for populations 1 and 2, respectively. In panels a–d, the contrasts between columns (a, c vs. b, d) and between rows (a, b vs. c, d) demonstrate significant genotype × environment (G × E) interactions due to the amount ($|D_1 - D_2|$) and angular direction ($\theta_{1,2}$) of phenotypic change, respectively. Panels b–e exhibit significant G × E interactions due to $|D_1 - D_2| > 0$, $\theta_{1,2} > 0$, or both.

phenotypic plasticity, accounting for trait covariation (e.g., Langerhans and DeWitt 2002). To visualize such differences, one can project multivariate G × E means onto the first two or three principal component axes of the multivariate set of trait values (Fig. 2), providing a rough interpretation of the significance of G, E, and G × E effects. However, this approach does not test specific hypotheses regarding the attributes of phenotypic change for multivariate reaction norms. Specifically, a significant G × E effect in MANOVA does not indicate

whether genetic differences in phenotypic plasticity occur because of differences in the amount of phenotypic change (Fig. 2b), differences in the direction of trait covariation (Fig. 2c), or both (Fig. 2d; as pointed out by Schlichting [1986]). Subsequent analyses are needed to test for differences in multivariate reaction norm magnitude and direction (two-state analysis; e.g., Collyer and Adams 2007).

Here we resolved this issue by describing reaction norms as multivariate vectors possessing magnitude and

direction in multivariate trait space. Reaction norms for multivariate data can be calculated as vectors (between two phenotypic states) that describe the difference in multivariate $G \times E$ means in more than two dimensions (Collyer and Adams 2007). Such vectors have two properties: magnitude (the amount of phenotypic change between two environments) and direction (the way traits covary between two environments; Fig. 2). Reaction norm vectors for two populations can be compared for these properties by (1) a contrast in their vector length ($|D_1 - D_2|$), and (2) the angle between vectors ($\theta_{1,2}$).

For a given population, the multivariate reaction norm is defined as a column vector of differences between phenotypic means in environments i and j, $\Delta\mathbf{X} = \overline{\mathbf{X}}_i - \overline{\mathbf{X}}_j$, where $\overline{\mathbf{X}}_i$ and $\overline{\mathbf{X}}_j$ are vectors of trait means in environment i and j, respectively. The Euclidean distance of a population's reaction norm is calculated as $D = (\Delta\mathbf{X}^T \Delta\mathbf{X})^{1/2}$ (where $\Delta\mathbf{X}^T$ is the transpose matrix of $\Delta\mathbf{X}$; Fig. 2). Given two populations (denoted 1 and 2), the similarity in orientation of their reaction norm vectors is described by their vector correlation (Schluter 1996), which is the inner product of the two vectors scaled to unit length:

$$ VC_{1,2} = \left[\frac{\Delta\mathbf{X}_1}{D_1}\right]^T \left[\frac{\Delta\mathbf{X}_2}{D_2}\right]. $$

The angle between vectors, $\theta_{1,2}$, is the arccosine of the vector correlation, $\theta_{1,2} = \cos^{-1}(VC_{1,2})$. Small angles imply similar orientation.

We used this approach of decomposing multivariate reaction norms into attributes of magnitude and direction for comparison of loosestrife phenotypic plasticity in life history traits between native and invasive provenances. Plants from both provenances were raised in four experimental environments that varied in water and nutrient levels (see *Methods: Seed collection* and *Common garden experiment*).

METHODS

Seed collection

We included three European populations (native provenance) and three North American populations (invasive provenance) to contrast native vs. invasive populations. In fall 2002, collections of seeds of *Lythrum salicaria* were obtained from three populations in North America (Little South Storm Lake, Iowa [42°38′ N, 95°13′ W]; Shell Rock, Minnesota [43°32′ N, 93°15′ W]; and Fayetteville, New York [42°49′ N, 76°49′ W]) and three populations from Germany (Schollener See [G1, 52°39′ N, 12°11′ E]; Strodehne [G2, 52°45′ N, 12°11′ E] in the state of Brandenburg; and Meißendorfer Teiche [G3, 52°43′ N, 9°50′ E] in the state of Saxony). Three populations from each provenance were chosen to represent a range of nutrient and moisture conditions found naturally in the field. We randomly chose seeds from a bulk sample of a large number of plants within

each population. As a consequence, our measurements of phenotypic plasticity reflect variability at the population level with three populations as replicates within each provenance.

Common garden experiment

Comparative studies of differences in plasticity between plants and populations are typically conducted in a single common garden. Although reciprocal transplant studies involving multiple field sites also have advantages in the study of invasiveness (Maron et al. 2004), we have conducted a common garden experiment because it enables us to better control and quantify environmental variation and to interpret the causal basis of phenotypic change. Our experiment was conducted in the forestry greenhouse at Iowa State University, Ames, Iowa, USA. Seeds from the six source populations were planted on 20 May 2004. After 20 days of growth, we selected 40 seedlings (2–4 cm in height) from each population, restricting initial size to reduce variation in traits related to germination and pre-transplant growth (i.e., variation due to maternal effects). Seedlings were individually transplanted into plastic pots (30 cm diameter × 25 cm depth) on 9 June 2004, and placed into plastic wading pools (1.4 m diameter × 30 cm depth). The pots were filled with Sunshine LC1 potting soil (Sun Gro Horticulture Canada, Seba Beach, Alberta, Canada), which has an initial nutrient charge that would correspond to high nutrient levels in natural wetland soils, approximately three times the average content of nitrogen as reported in Bridgham et al. (1996) and Bedford et al. (1999). However, the effect of this over the course of the experiment would be minimal, as the initial nutrient charge is highly water soluble, provides the equivalent of approximately one application of a liquid fertilizer, and needs to be supplemented by regular fertilizer treatments soon after potting. (Sun Gro Horticulture has provided the authors with proprietary information regarding average levels of the nutrient charge in LC1, through a personal communication.)

We applied a split-plot design consisting of five complete blocks, with four wading pools within each block. Each wading pool in a block contained one of four environmental treatment combinations: (1) low water, low nutrient ($W_L N_L$), (2) low water, high nutrient ($W_L N_H$), (3) high water, low nutrient ($W_H N_L$), and (4) high water, high nutrient ($W_H N_H$). Two plants from each population were put in each wading pool in each block (12 plants per pool). The total number of experimental units for this experiment was 5 blocks × 4 treatment combinations = 20 units, with 6 populations × 2 plants = 12 observations per experimental unit. Pots in the high water treatments were kept at saturation, similar to standing water conditions in lakes and ponds. Low water treatments are comparable to drier, upland conditions, where plants were watered every other day,

letting the soil soak for a few hours, after which the soil was allowed to dry.

In the low nutrient treatment no fertilizer was applied, whereas in the high nutrient treatment 100 g of slow-release 14:14:14 N:P:K Osmocote (The Scotts Company, Marysville, Ohio, USA) was applied once to a pot at the beginning of the experiment (N, 2481 mg/kg soil; P, 563 mg/kg soil; K, 1320 mg/kg soil). The Osmocote applied in the high nutrient treatment followed the manufacturer's recommendation for amount and rate (one application for a 3–4 mo growing period) to be used in producing high fertilization levels (The Scotts Company). As a consequence, the low and high nutrient treatments were designed to be a reasonable approximation of low and high soil nutrient levels, respectively, which would be encountered under natural field conditions (cf. Bridgham et al. 1996, Thormann and Bayley 1997, Bedford et al. 1999).

On 16 August 2004, after 92% of the plants had initiated flowering, they were harvested. As a perennial herb, purple loosestrife usually takes from four to five years to be competitively dominant under field conditions (Weiher et al. 1996, Mal et al. 1997b). However, as they begin to increase ramet production from the second year of growth (Mal et al. 1997b) it may be important to test whether native and invasive genotypes exhibit genetically based differences during the first year of growth in response to varying environmental conditions. We randomly chose wading pools across blocks for harvesting to minimize experimental variation due to growth during the harvesting period. The following six traits were measured for each experimental individual: (1) height, (2) number of secondary branches, (3) number of stems originating from the rootstock, (4) total number of flowers per plant, (5) aboveground biomass, and (6) belowground biomass. To determine (4), we haphazardly sampled five flower stalks from each population and treatment combination (one flower stalk from each block) to count the number of flowers per unit length of flower stalk. We measured total length of flower stalks per plant for each individual. Total number of flowers per plant was then calculated by multiplying the number of flowers per unit length by total length of flower stalk per plant, for each population and treatment combination. To determine aboveground and belowground biomass, plants were divided into respective parts and dried in an oven for 24 hours at 60°C to constant weight.

Application of multivariate analyses

We analyzed the phenotypic plasticity of purple loosestrife populations from the native and invasive provenance for the four environmental treatment combinations. To avoid pseudoreplication, the data for the two replicate plants in each treatment were averaged prior to analysis (creating 120 total observations). Two of these observations were removed from the study because they were outliers due to individuals showing retarded growth. Data were log-transformed to create variables that were more normally distributed to satisfy assumptions of our analytical methods.

We analyzed our data in three ways: PCA, mixed-model MANOVA, and the two-state multivariate analysis of phenotypic plasticity. First, we conducted PCA on the correlation matrix calculated from log-transformed variables to reduce the dimensionality of data because of trait variation due to allometry, resulting in six PC axes. The PCA indicated that four phenotypic traits (number of secondary branches, number of stems originating from rootstock, aboveground biomass, and belowground biomass) were all strongly, positively associated with PC1, thus indicating a collinear relationship of PC1 with plant "size." Size effects may obscure the actual effects of provenance and environment in which we are interested. To explain the phenotypic responses holding the size effect constant, we excluded PC1 and made a new data set composed of five principal components (PC2 through PC6), hereafter referred to as the "reduced" data set, in contrast to the "full" data set (raw data).

Second, we performed MANOVA on the full and reduced data sets to test for differences in phenotype due to the effects of native and invasive provenance (G effect), nutrient and water conditions (E effects), and their interactions (G × E effect). Differences among blocks (main plot) were analyzed using the block × environment (subplot) interactions as error terms. A significant G × E interaction indicates that plants from the different provenances differ in their multivariate phenotypic plasticities.

Finally, using the two-state methods of Collyer and Adams (2007), we calculated multivariate reaction norms for all six possible pairwise comparisons of G × E means (estimated as least-squares means from the MANOVA model) within provenance. The significance of each reaction norm magnitude was determined by a Hotelling (1931) multivariate T^2 test by converting reaction norm Euclidian distances to squared Mahalanobis (1936) distances, using the model error variance/covariance matrix from the MANOVA (see Legendre and Legendre [1998] for test details). Subsequently, reaction norms were compared in magnitude and direction using a permutation procedure that preserves overall genetic (G) and environmental (E) effects, but assumes a null model of Var(G × E) = 0 (see Collyer and Adams [2007] for statistical details).

The permutation procedure uses a two-stage operation. In the first stage, a linear model that lacks the G × E effect is used to generate predicted values and residuals. These residuals are the permuted units, and with every iteration of the permutation procedure, new values for the response variables are created from the predicted values and random residuals. In the second stage of the procedure, a linear model that contains the G × E effect is used to estimate least-squares treatment means. Thus, random reaction norms are calculated at each iteration,

TABLE 1.   PCA results (loadings) on the log-transformed full data set.

Variables	PC1	PC2	PC3	PC4	PC5	PC6
Height	0.103	0.713	−0.441	0.423	−0.264	−0.196
Branches	0.486	−0.288	0.285	0.106	−0.416	−0.645
Stems	0.455	0.277	0.397	0.266	0.698	−0.012
Flowers	0.269	−0.540	−0.662	0.342	0.284	0.023
Aboveground biomass	0.535	−0.005	0.100	0.061	−0.414	0.727
Belowground biomass	0.433	0.203	−0.344	−0.786	0.130	−0.130
Eigenvalue	3.248	1.494	0.613	0.407	0.179	0.059
Cumulative percentage of variance						
PC1–PC6	54.13	79.03	89.25	96.03	99.02	100.0
PC2–PC6†		54.28	76.57	91.35	97.86	100.0

† Calculated assuming that the total variance (PC2–PC6) is 100.

holding the overall G and E effects constant. We calculated pairwise contrasts and angles between native and invasive provenances for 4999 permutations of random vectors under the null hypothesis of Var(G × E) = 0. Along with observed values, this created empirical reference distributions of 5000 contrasts and angles from random vectors. We calculated the probability of finding, by chance, more extreme contrasts or angles than observed values from the empirical reference distributions created from random pairs of vectors.

PCA was performed with R (R Development Core Team 2005) and sums of squares cross products (SSCP) matrices were calculated with JMP (Sall et al. 2003) to perform the mixed-model MANOVA. The determinants of SSCP matrices were used to calculate Wilks' Λ. Permutation tests were run for both full and reduced data sets, using the Monte-Carlo analysis of PopTools (Hood 2005). To preserve the error rate of $\alpha = 0.05$ for the entire set of comparisons, we used sequential Bonferroni corrections for pairwise comparisons (Rice 1989).

RESULTS

PC1 (the principal axis of variation) and PC2 together explained 79% of the total variation in the full data set (Table 1). PC1 was positively associated with number of secondary branches, number of stems originating from the rootstock, aboveground biomass, and belowground biomass (plant "size" indicators); whereas PC2 was positively associated with plant height and negatively associated with number of flowers, representing a trade-off between growth and reproduction. PC3 was negatively associated with height and number of flowers. After removing the variance associated with the size effect of PC1, 76.6% of the total remaining variation was explained by PC2 and PC3 (Table 1). Whether variation due to size allometry was included (Fig. 3a) or excluded (Fig. 3b), differences in phenotype among environmental treatments were obvious.

The MANOVA results indicated that overall G, E, and G × E effects were significant for both the full and reduced data sets (Table 2). The only relative difference found between MANOVAs of the two data sets was related to G × E interactions involving water level (W effect), which were significant for the reduced data set

but not for the full data set. These contradictory results suggest that even though the differences in growth were similar for plants from native and invasive provenances across water treatments, each provenance had different phenotypic plasticities across the same water treatments when the effect of size allometry was removed.

All reaction norm magnitudes were significantly greater than zero ($P < 0.003$), but there were several differences in reaction norm magnitude between provenances (Table 3). The differences of reaction norm magnitudes between provenances for $W_L N_L − W_H N_L$ and $W_L N_H − W_H N_L$ were significant for both the full and reduced data sets (Table 3). In both cases, reaction norm magnitudes were greater for native plants than for invasive plants. This indicates that plants from the native provenance exhibited a greater amount of phenotypic change when water conditions changed under low nutrient conditions ($W_L N_L − W_H N_L$) or when water level and nutrient level changed in the opposite direction ($W_L N_H − W_H N_L$). Plants of the invasive provenance, in contrast, exhibited greater phenotypic change for the reaction norm for $W_L N_L − W_H N_H$, but only for the full data set (Table 3), indicating that invasive plants exhibited a greater growth size response between environments that differed in both water and nutrient level in the same way. The directional difference was significant for the $W_L N_H − W_H N_L$ and $W_H N_L − W_H N_H$ reaction norms for both the full and reduced data sets (Table 3), which indicates that plants from native and invasive provenances differed in the way phenotypic traits covaried under changing nutrient conditions (Table 2).

Taken together, our results indicate that native plants demonstrate greater phenotypic plasticity to changing water level, though when water level and nutrient level changed in the same direction, invasive plants demonstrate greater phenotypic plasticity in growth. The two provenances also differ in the way traits covary between environments that differ in nutrient levels ($W_L N_H − W_H N_L$ and $W_H N_L − W_H N_H$).

DISCUSSION

There are few comparative studies of phenotypic plasticity in native vs. invasive populations and little
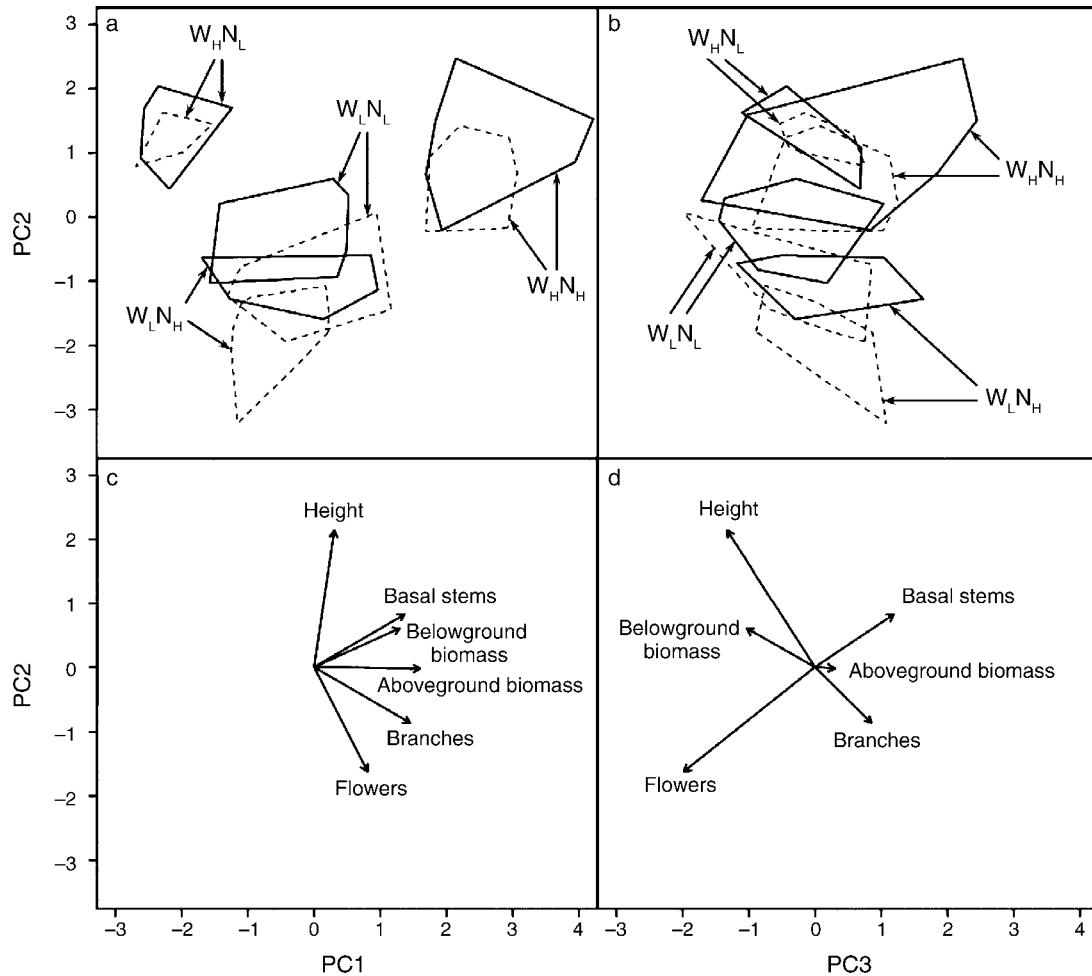
FIG. 3. Principal component analysis (PCA) plots of (a, c) PC1 vs. PC2 (including size effects), and (b, d) PC2 vs. PC3 (excluding size effects). Plots (a) and (b) include polygons that represent convex hulls for each provenance and treatment combination; solid and dashed lines represent invasive and native populations, respectively. Plots (c) and (d) include arrows that indicate the direction and length of trait vectors in the same PC space. Environmental treatments are: W_LN_L, low water, low nutrient; W_LN_H, low water, high nutrient; W_HN_L, high water, low nutrient; W_HN_H, high water, high nutrient.

consensus as to whether a distinct plastic response by invasive populations is related to invasiveness (Richards et al. 2006). About half of the studies examining this issue conclude that there is no difference in response, whereas the other half indicates that phenotypic plasticity predisposes invasive populations to be more aggressive than native populations (Bossdorf et al. 2005). Recent studies, in fact, have shown that within a species the interpretation of the importance of plasticity to invasiveness may vary depending upon the trait examined (e.g., Barney et al. 2005, Brock et al. 2005, Burns and Winn 2006). Further empirical studies contrasting phenotypic plasticity between native and invasive populations may increase our understanding of the process of invasion, especially when conducted under conditions that the populations normally experience in the field. Also, by extending the analysis of plasticity to incorporate a multivariate approach, some

of the problems recently identified in analyzing multiple traits in a univariate context may be mitigated (cf. Barney et al. 2005, Brock et al. 2005, Burns and Winn 2006).

Although there is no previous study comparing the magnitude and direction of multivariate reaction norms of purple loosestrife between native and invasive populations, previous studies of purple loosestrife have found genetic variation for plasticity to water and nutrient availability. In a field study by Edwards et al. (1998), fecundity of purple loosestrife was similar between United States and European populations in nutrient-poor habitats, but was significantly greater in United States populations in nutrient-rich habitats (cf. Burns and Winn 2006). In a study conducted on 11 purple loosestrife populations from different latitudes in Europe, Bastlová et al. (2004) reported significant latitude × nutrient and latitude × water interactions on

TABLE 2. MANOVA results for full and reduced data sets.

		Full data set				Reduced data set			
Source	Wilks' Λ	Λ_{obs}	F	df	P	Λ_{obs}	F	df	P
B	$\dfrac{\lvert BE \rvert}{\lvert BE + B \rvert}$	0.0067	4.816	24, 36.10	<0.0001	0.0160	4.643	20, 37.43	<0.0001
G	$\dfrac{\lvert \varepsilon \rvert}{\lvert \varepsilon + G \rvert}$	0.3656	25.734	6, 89.00	<0.0001	0.3811	29.229	5, 90.00	<0.0001
E	$\dfrac{\lvert \varepsilon \rvert}{\lvert \varepsilon + E \rvert}$	0.0028	97.765	18, 252.22	<0.0001	0.0344	39.623	15, 248.85	<0.0001
W	$\dfrac{\lvert \varepsilon \rvert}{\lvert \varepsilon + W \rvert}$	0.0972	137.757	6, 89.00	<0.0001	0.1377	112.696	5, 90.00	<0.0001
N	$\dfrac{\lvert \varepsilon \rvert}{\lvert \varepsilon + N \rvert}$	0.0599	232.963	6, 89.00	<0.0001	0.1577	96.165	5, 90.00	<0.0001
W × N	$\dfrac{\lvert \varepsilon \rvert}{\lvert \varepsilon + WN \rvert}$	0.0713	193.278	6, 89.00	<0.0001	0.3032	41.375	5, 90.00	<0.0001
G × E	$\dfrac{\lvert \varepsilon \rvert}{\lvert \varepsilon + GE \rvert}$	0.6194	2.586	18, 252.22	0.0006	0.6530	2.769	15, 248.85	0.0006
G × W	$\dfrac{\lvert \varepsilon \rvert}{\lvert \varepsilon + GW \rvert}$	0.8745	2.129	6, 89.00	0.0576	0.8755	2.560	5, 90.00	0.0326
G × N	$\dfrac{\lvert \varepsilon \rvert}{\lvert \varepsilon + GN \rvert}$	0.7985	3.744	6, 89.00	0.0023	0.8377	3.489	5, 90.00	0.0063
G × W × N	$\dfrac{\lvert \varepsilon \rvert}{\lvert \varepsilon + GWN \rvert}$	0.8822	1.980	6, 89.00	0.0768	0.8856	2.325	5, 90.00	0.0492
B × E	$\dfrac{\lvert \varepsilon \rvert}{\lvert \varepsilon + BE \rvert}$	0.2755	1.820	72, 490.02	<0.0001	0.3408	1.832	60, 425.21	0.0004

Notes: Sums of squares and cross product matrices (SSCP) are denoted as: B, block; G, provenance (native vs. invasive); E, environment; W, water; N, nutrient; and ε, error. Water (W) and nutrient (N) are subcategories of environment (E). Values of $P < 0.05$ are significant.

some growth characteristics, where size-related traits were negatively correlated with latitude. These findings support the idea that purple loosestrife from different geographic provenances exhibit different amounts of plasticity in response to changes in water or nutrient conditions. These studies used univariate statistical approaches to analyze phenotypic plasticity, providing useful insights into the invasion biology of purple loosestrife. However, their interpretation is limited to an exploration of the presence or absence of G (genotypic), E (environmental), or G × E effects on single traits. By adding a multivariate component to the analysis, we hoped to gain a deeper understanding of the integrated response among traits to changing environmental conditions.

The two-state multivariate analysis applied in our study is advantageous for testing hypotheses concerning population differences in both the amount and direction of phenotypic plasticity in a multivariate trait space. It offers the additional benefit, compared to MANOVA, of interpreting the meaningful aspects of phenotypic change that produce a significant G × E interaction.

TABLE 3. Summary table analyzing the difference in vector magnitude and direction on full and reduced data sets.

	Full data set					Reduced data set				
	Euclidean distance					Euclidean distance				
Reaction norm vectors	N	I	P_D	$\theta_{N,I}$	P_θ	N	I	P_D	$\theta_{N,I}$	P_θ
$W_L N_L - W_L N_H$	0.21	0.19	0.7560	75.88	0.1434	0.09	0.07	0.6702	73.97	0.1368
$W_L N_L - W_H N_L$	**1.27**	0.96	**0.0116**	10.91	0.0998	**0.40**	0.27	**0.0062**	10.76	0.2098
$W_L N_L - W_H N_H$	0.80	**0.99**	**0.0022**	9.59	0.4662	0.15	0.18	0.6746	20.48	0.1274
$W_L N_H - W_H N_L$	**1.42**	1.07	**0.0062**	**19.33**	**0.0014**	**0.48**	0.31	**0.0012**	20.50	**0.0056**
$W_L N_H - W_H N_H$	0.90	0.93	0.6604	12.62	0.2650	0.22	0.17	0.4054	13.21	0.3046
$W_H N_L - W_H N_H$	1.67	1.68	0.8820	**14.24**	**0.0066**	0.29	0.23	0.0368	**34.80**	**0.0120**

Notes: Symbols represent: N, native provenance; I, invasive provenance; $W_L N_L$, low water, low nutrient; $W_L N_H$, low water, high nutrient; $W_H N_L$, high water, low nutrient; $W_H N_H$, high water, high nutrient. P_D represents the empirical probability that the absolute difference in the magnitude of random reaction norms is larger than the observed difference. $\theta_{N,I}$ indicates the observed angle between reaction norms of native (N) and invasive (I) provenances. P_θ represents the probability that the angle between pairs of random reaction norms is larger than that of the observed angle. Bold values indicate where significant differences in magnitudes or angles occurred, after sequential Bonferroni correction to maintain $\alpha = 0.05$.

Description and comparison of magnitude and direction in phenotypic change vectors provides greater insight into the nature of differences in phenotypic plasticity between native and invasive species.

In our study comparing native vs. invasive populations of purple loosestrife, we found significant differences in multivariate phenotypic plasticity for comparisons between low and high water treatments within low nutrient levels, between low and high nutrient levels within high water treatments, and for comparisons that included both a water and nutrient level change (Table 3). The significant reaction norm differences occurred because of differences in the magnitude (D) of phenotypic plasticity in response to water level change and different directional responses (θ) to nutrient level change, which were detected via the two-state multivariate methods applied to this study. Based on the statistical results for reaction norm comparisons (Table 3), the covariation of original variables and PCs (Fig. 3), and plots of reaction norms projected onto PCs (Fig. 4), we are able to draw several conclusions concerning differences in the nature of plasticity in native vs. invasive populations. This served to extend the understanding gained through the more traditional univariate approaches (e.g., Fig. 1, and related analyses; Edwards et al. 1998, Bastlová et al. 2004).

First, given low nutrient availability, conditions for growth were better under low water conditions ($W_L N_L$ vs. $W_H N_L$), where all of the measured traits were greater in amount, except for height (Fig. 1). For both the full and reduced data sets, reaction norms associated with changing hydrology under low nutrient conditions differed significantly in magnitude, but not direction ($W_L N_L - W_H N_L$; Table 3, Fig. 4a, b). This indicates that the general growth response to changes in hydrology was the same for plants from both provenances, but the amount of change was significantly greater for plants of native provenance (Table 3). This is most obvious in the projection of the reaction norms on PC2, an axis most associated with variation in height and flower production (cf. Table 1, Figs. 1, 3, 4a, b). There is a trade-off between height and flower production along PC2, where invasive populations tend to increase height while native populations are likely to increase flower production in response to changing water levels with low nutrient conditions (Figs. 1a, d, and 3).

Second, under constant flooding, conditions for growth were better with high nutrient availability ($W_H N_L$ vs. $W_H N_H$), where, with the exception of height, all of the measured traits were greater in amount (Fig. 1). For both the full and reduced data sets, reaction norms associated with these two treatment combinations differed significantly in direction, but not magnitude ($W_H N_L - W_H N_H$; Table 3, Fig. 4c, d). This directional difference between native and invasive plants represents a difference in the covariation of traits in response to changes in environmental conditions. From $W_H N_L$ to

$W_H N_H$, the reaction norm of native plants is oriented approximately in the direction of increased number of branches (Figs. 4d, 3d, and 1b), while the reaction norm of invasive plants is directed toward increased aboveground biomass (Figs. 4d, 3d, and 1e). In interpreting this result it must be kept in mind that the PC scores were obtained through an analysis of the correlation matrix, which places equal emphasis on all six traits. This may downplay the impact of traits exhibiting the greatest response to the changing treatment conditions, but takes into account significant trends in the plastic response of all six traits.

Third, reaction norms in which water and nutrient levels changed in opposite directions differed in both magnitude and direction for both data sets ($W_H N_L - W_L N_H$ in Table 3, Fig. 4c, d). The change in magnitude was greatest for the native provenance (Table 3), primarily in the direction of PC2 (Fig. 4e, f). An inspection of trait loadings on PC2 suggests that native plants should exhibit a greater change in flower production and height in response to the shift in environmental conditions between $W_H N_L$ and $W_L N_H$ (Table 2). A direct examination of mean trait responses indicates a trade-off between height and flower production along PC2, where native plants suffer reduction in height coupled with a disproportionate gain in flower production as compared to invasive plants, in response to low water, high nutrient conditions (Fig. 1a, d). Native plants also show a decrease in allocation to belowground biomass, which could represent a decrease in allocation to growth for the next growing season. In contrast, the invasive plants increased allocation to belowground biomass under the same conditions. These differential responses result in a significant difference in direction between reaction norms and represent a genetic difference in the response by the invasive vs. native provenance to the underlying environmental conditions. It is interesting to note that the $W_H N_L$ treatment could be interpreted as representing the conditions most likely experienced by plants in native habitat, whereas the $W_L N_H$ treatment most closely approximates the situation of plants expanding into more mesic conditions associated with high nutrient inputs, as is the case for some North American populations (K. A. Moloney, *personal observation*). Greater allocation of resources to fecundity and less to belowground storage under low water conditions by the native plants could represent a stress response that is not being expressed by the invasive plants (cf. Pigliucci and Schlichting 1998).

It is interesting to contrast the previous result with the reaction norms between the treatments where water and nutrient levels change in the same direction ($W_L N_L - W_H N_H$). For this reaction norm, the only significant effect was a difference in magnitude for the full data set, representing a generalized response in the same direction for both provenances. In this case, the invasive plants had a greater response than did the native ones. All of
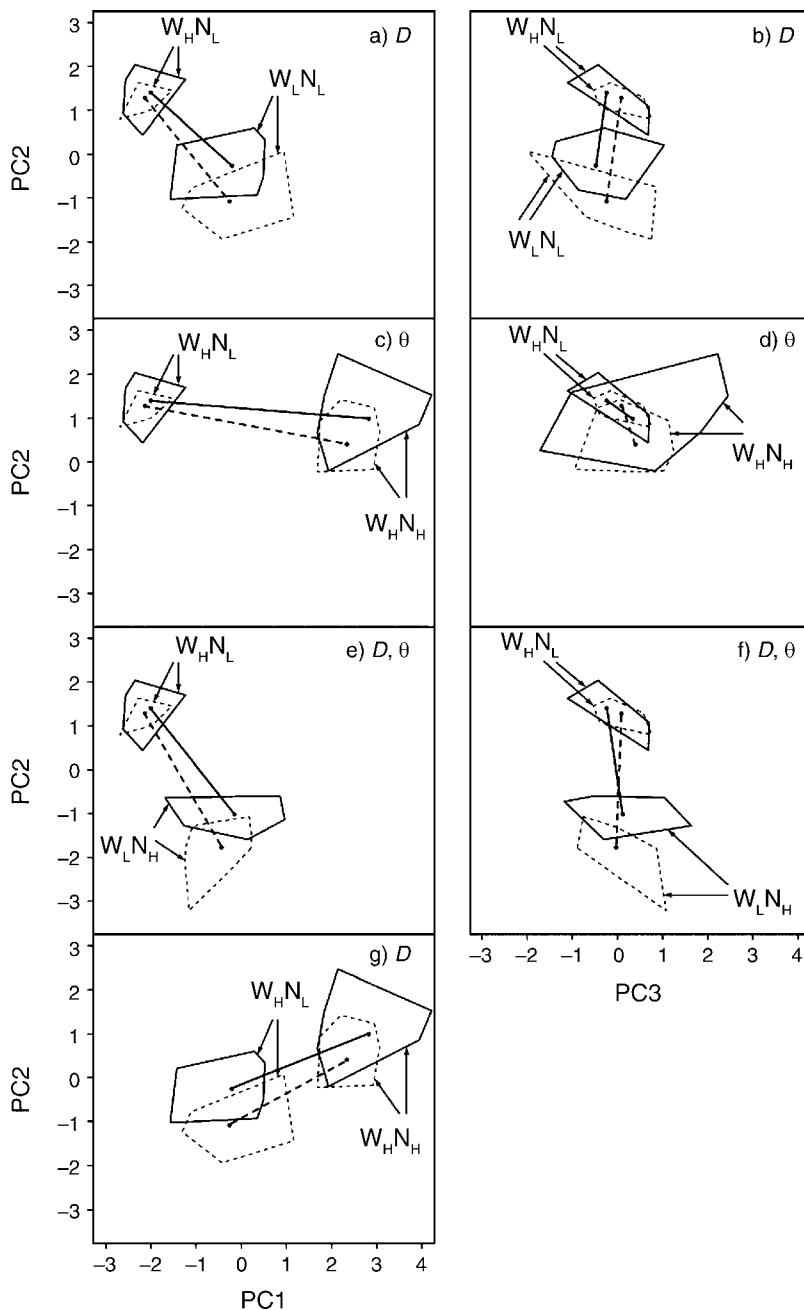
FIG. 4. PCA plots showing reaction norms significantly different for D and/or θ between native plants and invasive plants. Solid and dashed lines represent invasive and native populations, respectively. Left-hand panels are for PC1 vs. PC2 (including size effects), and right-hand panels are for PC2 vs. PC3 (excluding size effects). Environmental treatments are: $W_L N_L$, low water, low nutrient; $W_L N_H$, low water, high nutrient; $W_H N_L$, high water, low nutrient; $W_H N_H$, high water, high nutrient.

the traits, except height, responded by increasing in amount with a concomitant increase in water and nutrient availability. The response to an increase in water levels and a corresponding decrease in nutrients (or vice versa) was more complex, representing trade-offs that differed among traits and provenances.

In addition to the environmental conditions resulting in significant differences between native and invasive populations, it is also interesting to consider the two sets of conditions under which differences in phenotypic plasticity were not observed. Under conditions of low water availability but shifting nutrient status ($W_L N_L -$ $W_L N_H$), phenotypic plasticity was minimal for both provenances and data sets (full and reduced; Table 3). This indicates that when water availability is low, the potential for plastic response is limited in both native

and invasive populations, regardless of variation in nutrient availability. In contrast, under conditions of high nutrient availability there was a large plastic response to change in water availability ($W_LN_H - W_HN_H$), though this response did not differ in magnitude or direction for either provenance or data set (Table 3). This implies that when nutrients are not limiting, both native and invasive populations can be expected to exhibit similar patterns of phenotypic change in response to environmental variation in water availability.

In general, our results indicate that native and invasive purple loosestrife plants exhibit different patterns of plastic responses, depending on specific environmental changes in water and nutrient levels. However, questions can be raised as to whether this difference is accompanied with an increase in plant function and fitness. Greater magnitude of plastic responses does not necessarily mean greater fitness and adaptation (Richards et al. 2006). Native plants produced more flowers in response to low water availability (Fig. 1d), but invasive plants were taller with greater aboveground biomass over all treatment combinations (Fig. 1a, e; and as indicated in Fig. 3, particularly with respect to PC2). Our results agree with Bastlová and Květ (2002) who found that nonnative purple loosestrife plants allocate more biomass to aboveground shoots while native plants allocate more to reproductive parts. In addition, Mal et al. (1997a) reported that populations invading Canada significantly increased clonal growth in response to increased water or nutrient levels, but flower production didn't increase. This may reflect a difference in reproductive strategy between native and nonnative populations in the first season of growth; native populations are primarily producing flowers whereas invasive populations rely on clonal reproduction and increased allocation to storage in the roots.

Considering that the onset of flowering in invasive plants is approximately ten days later than in native plants (Bastlová and Květ 2002), it appears that invasive plants have developed a strategy of a more extended period of vegetative growth before flowering, which might potentially contribute to increased fitness in successive years. In the first year of growth by a perennial plant, it might be advantageous to allocate more energy to vegetative growth and storage rather than sexual reproduction. Preliminary studies suggest that invasive purple loosestrife plants have a longer lifespan than native plants (H. Dietz and K. A. Moloney, *unpublished data*). A long-term study of the type presented here may be required to determine if perennial invasive plants exhibit more adaptive plasticity in the long run than native plants.

In any case, our results support the argument that invasiveness of purple loosestrife is closely associated with the interaction of high levels of soil nutrient and a flooding water regime. Invasive populations exhibit significantly greater shoot biomass than native populations in nutrient-rich field conditions with a standing water regime (Edwards et al. 1998). Weiher et al. (1996) found that purple loosestrife plants in northeast North America establish and subsequently dominate in local communities when soil fertility is high, in either standing water or a seasonally flooded water regime. However, establishment was lowest when soil fertility was low in seasonally flooded water regimes. In addition, purple loosestrife generally grows taller under high moisture conditions (Lempe et al. 2001, Shadel and Molofsky 2002). Our results agree with these studies, in that height of invasive plants is greatly increased in high water conditions, and most of the other traits are greatly increased in high nutrient, high water conditions.

Although purple loosestrife is an aggressive invader in North America, it is mostly found in permanently or periodically flooded habitats, while these plants actually have a wider ecological range in Europe (Bastlová-Hanzélyová 2001). However, it has been repeatedly reported that invasive populations tend to be tall and vigorous in growth, often forming high-density mono-culture stands, while native populations are composed of more widely scattered plants of generally smaller stature (Blossey and Nötzold 1995, Edwards et al. 1998, Bastlová-Hanzélyová 2001, Bastlová and Květ 2002). Our results indicate that invasive plants exhibit greater plasticity than native plants when water and nutrient levels increase simultaneously ($W_LN_L - W_HN_H$, Table 3), especially with respect to an increase in height and aboveground biomass (Fig. 1). In contrast to native plants, invasive purple loosestrife plants may have evolved a greater ability to take advantage of high nutrient levels, produced from disturbances and human activities, especially under flooded conditions. This may help explain the accumulation of invasive potential in the first growing season and the development of aggressiveness in subsequent years in the invaded range of North America.

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