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Comparing the genetic architecture and potential response to selection of invasive and native populations of reed canary grass

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Abstract

Evolutionary processes such as migration, genetic drift, and natural selection are thought to play a prominent role in species invasions into novel environments. However, few empirical studies have explored the mechanistic basis of invasion in an evolutionary framework. One promising tool for inferring evolutionarily important changes in introduced populations is the genetic variance–covariance matrix (G matrix). G matrix comparisons allow for the inference of changes in the genetic architecture of introduced populations relative to their native counterparts that may facilitate invasion. Here, we compare the G matrices of reed canary grass (*Phalaris arundinacea* L.) populations across native and invasive ranges, and between populations along a latitudinal gradient within each range. We find that the major differences in genetic architecture occur between populations at the Northern and Southern margins within each range, not between native and invasive populations. Previous studies have found that multiple introductions in introduced populations caused an increase in genetic variance on which selection could act. In addition, we find that differences in the evolutionary potential of *Phalaris* populations are driven by differences in latitude, suggesting that selection also shapes the evolutionary trajectory of invasive populations.

Introduction

The study of invasive species is providing major insights into the evolutionary mechanisms driving species adaptation to new environments and geographic range expansion (Facon et al. 2006). A large interest has been devoted to understand how the history of species introduction and subsequent genetic admixture shapes patterns of genetic diversity and evolutionary potential in the invasive range of introduced species (Kolbe et al. 2004; Lavergne and Molofsky 2007; Dlugosch and Parker 2008). However, few empirical studies have moved toward an evolutionary framework to explain and predict how evolution will drive future biological invasions (Facon et al. 2006). Such a framework should not only take into account patterns of standing genetic variance in adaptive traits, as it has been traditionally advocated since Fisher (1930), but also focus on the patterns of genetic architecture for multi-trait phenotypes and include traits relevant to species interactions (for a review on such an eco-evolutionary approach, see Lavergne et al. 2010a).

Contrary to the expectation that introduced populations should have low levels of genetic variance because of bottlenecks during founding events (Mayr 1942; Lande 1992), recent studies have shown that invasive populations may retain high levels of genetic variance, especially when invasives derive from multiple source populations (Kolbe et al. 2004; Lavergne and Molofsky 2007). An important step in identifying the evolutionary processes that facilitate biological invasions is to compare the underlying genetic architecture of native and invasive populations for multiple traits in concert, instead of comparing the amount of genetic variance for several traits in isolation (Bacigalupe 2009; Colautti et al. 2010). Simply

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identifying high levels of additive genetic variation for 'invasion traits' does not accurately determine whether an introduced population can respond to selection in a new environment and become a successful invader. Genetic correlations among traits may constrain phenotypic evolution and restrict the evolutionary trajectory of a population along major multivariate trait axes (Schluter 1996). Thus, in addition to measuring the amount of additive genetic variance available in a population on which selection can act, it is important to identify the underlying genetic architecture (i.e. trait correlations that shape the response to selection) of traits important for invasion. Understanding the genetic architecture of key life history traits can provide novel insights into how introduced populations will be able to adapt and spread beyond their current geographic range (Colautti et al. 2010).

One promising tool for understanding the role of genetic architecture in microevolutionary dynamics is the G matrix (**G**). G matrices provide a multi-trait summary of the amount of heritable variance in a population, and, at the same time, represent the genetic constraints on phenotypic evolution (Steppan et al. 2002). As a key variable in the multivariate breeder's equation ($R = \mathbf{GP}^{-1}$ s), **G** is a useful means of predicting the outcome of multivariate selection. For example, statistical comparisons of **G** in native and invasive populations would help determine whether invasion alters a population's genetic architecture (i.e., alters the structure of **G**). Differences in **G** could arise through population bottlenecks via a decrease in additive genetic variance (Whitlock and Fowler 1999), an increase in genetic variance via conversion of nonadditive to additive genetic variance (Goodnight 1988), or by changes associated with multiple introductions from different native populations. Bottlenecks or multiple introductions may breakdown genetic correlations between traits that constrain phenotypic evolution, thus facilitating adaptation and subsequent invasion in novel environments. By contrast, identifying similarities in **G** between native and invasive populations could identify important shared evolutionary constraints that would restrict an introduced population from successfully invading a new environment.

Recently, studies have highlighted the fact that comparisons of genetic variance in native and invasive populations are often confounded by clinal (e.g. latitudinal) variation in introduction history, genotype by environment interactions, or differences in natural selection (Bacigalupe 2009; Colautti et al. 2009, 2010). Thus, comparisons of genetic variance in native and invasive populations must account for fluctuations in the amount and structure of genetic variance across each range.

Here, we compare the genotypic variance–covariance matrices (**G**) of native and invasive populations of reed canary grass (*Phalaris arundinacea* L.) to determine whether changes in **G** explain the invasive potential of introduced North American populations. Our approach is novel in that we compare variation in genetic architecture across native and invasive populations at the Northern and Southern margin of each range, as well between populations along a latitudinal gradient within each range. Invasive populations of *Phalaris* in North America are the result of multiple, unmonitored introductions from native European populations (Merigliano and Lesica 1998; Lavergne and Molofsky 2007). Subsequent recombination between European strains has increased genetic variance in introduced populations and potentially resulted in increased evolutionary potential for vegetative spread (Lavergne and Molofsky 2007; Lavergne et al. 2010b).

The goal of our study was to compare the genetic architecture of native and invasive populations of reed canary grass and infer the evolutionary processes that may have facilitated invasion. We first tested whether changes in the genetic architecture of invasive populations could alter their evolutionary trajectories relative to native populations. To do this, we compared overall G matrix patterns and the predicted response to selection in native and invasive populations using the following: (i) hypothetical selection scenarios acting on traits predicted to be important for invasion into native plant communities and (ii) selection differentials measured in a selection experiment. In addition to native vs invasive comparisons, we also compared **G** between Northern and Southern populations within the native and invasive ranges to determine whether differences in selection (e.g., selection imposed by climate variation experienced by Northern and Southern populations) could influence the structure of **G**, which would suggest that selection along environmental gradients has shaped native and invasive *Phalaris* populations.

Methods

Study species

Phalaris arundinacea L. is a tall, cool-season perennial grass (Hodgson 1968; Comes 1971) native to Eurasia (Marten 1985). It grows in wetland habitats and wet meadows throughout Europe (Conchou and Patou 1987; Klimesova and Cizkova 1996). Introduction to North America from Europe occurred shortly after 1850 and has since spread throughout the United States where it is classified as a pest species in nine states (Hodgson 1968; Comes 1971; Marten 1985; USDA and NRCS 2001). *Phalaris* can take over wetlands, clog waterways, and dominate sections of pastures (Marten 1985).

Greenhouse experiment

We estimated the genotypic variance and covariance for native and invasive *Phalaris* populations using a greenhouse experiment. From 210 genotypes identified through isozyme analysis (Lavergne and Molofsky 2004), 49 invasive (Vermont, 44°28′N, 73° 9′W, $N = 23$; North Carolina, 35°19′N, 83° 38′W, $N = 26$) and 41 native (Czech Republic, 49°00′N, 14° 46′E, $N = 28$; France, 43°37′N, 3° 52′E, $N = 13$) genotypes were chosen for a greenhouse experiment. Briefly, four clones of each genotype were grown in a common greenhouse environment (22–25°C diurnal temperature), and emergence time, tiller number, maximum tiller height, above-ground biomass, below-ground biomass, and total biomass were measured for each clone (see Lavergne and Molofsky 2007 for complete methods).

G matrix comparison: hypothetical selection scenario

We estimated the phenotypic (**P**) and genotypic (**G**) variance–covariance matrices of *Phalaris* populations to identify differences in genetic architecture between Northern native and invasive populations (Czech Republic vs Vermont), Southern native and invasive populations (France vs North Carolina), and among Northern and Southern populations within each range (Czech vs France and Vermont vs North Carolina). It is important to emphasize that we estimated **G** using genotypic variances (which includes additive variance, dominance variance, and interaction variance; Hartl 2000) rather than using only additive genetic variance only as is often the case in G matrix studies. The P matrix and G matrix for each population was estimated for three phenotypic traits measured in the greenhouse experiment: emergence time, height, and tiller number, using separate multivariate analyses of variance (MANOVA; Anderson 2003; Tables S1–S4). These traits were chosen because they likely play a major role in the invasion success of reed canary grass (Gifford et al. 2002; Lavergne and Molofsky 2007; Lavergne et al. 2010a,b). Specifically, emergence date has been shown to confer an adaptive advantage in perennial plant communities (Verdu and Traveset 2005), and vegetative height or vegetative size is directly involved in competitive hierarchies (Keddy et al. 2002). Because of this, we hypothesized that *Phalaris* populations in the invasive North American range experience directional selection for vegetative traits increasing competitive ability in the context of North American wetlands communities. Specifically, we hypothesized selection for decreased emergence time, increased height, and increased tiller number.

We compared G matrices between native and invasive populations at the Northern range margins, native and invasive populations at Southern range margins, and between Northern and Southern populations within each range. Specifically, we tested the hypotheses that the genetic architecture of reed canary grass is shaped by (i) differences between the native versus invasive ranges (because of invasion history) and (ii) differences in latitude within each range separately.

To compare **G** across populations or ranges, we used a recently described G matrix comparison statistic, the selection skewers method (Calsbeek and Goodnight 2009). The selection skewers method determines whether the evolutionary trajectories of two populations are significantly different, because of underlying differences in their genetic architecture. In contrast to other G matrix comparison statistics (which compare the overall structure of two G matrices), the selection skewers method identifies biologically relevant changes in **G**. The selections skewers method applies the breeder's equation to determine whether the response to a hypothetical selection scenario (e.g. selection in the introduced range) would be significantly different in two populations because of underlying differences in **G**.

We used the selection skewers method for all G matrix comparisons and imposed three hypothetical selection scenarios in each analysis: (i) selection for decreased emergence time, (ii) selection for increased height, and (iii) selection for increased tiller number. The truncation point for all selection scenarios was set so that 25% of individuals were selected. We then tested whether the response to selection would be significantly different in the populations because of differences in their G matrices. Complete methods for the selection skewers analysis are provided elsewhere (Calsbeek and Goodnight 2009). Briefly, we applied hypothetical selection scenarios to datasets from both populations by truncation selection and then calculated selection differentials (s) for each population. We multiplied selection differentials from each population by the inverse of its respective phenotypic variance–covariance matrix ($\mathbf{P}^{-1}$) and G matrix (**G**) to calculate the response (R) to the hypothetical selection scenario. We then calculated the selection skewers statistic as the vector correlation of response vectors:

$$\mathrm{Corr}(\nu_1, \nu_2) = \frac{\nu_1^T \nu_2}{\sqrt{(\nu_1^T \nu_1)(\nu_2^T \nu_2)}}$$

where ν_1 is the response to selection vector for the first population in the comparison, and ν_2 is the response for the second.

In addition to the selection skewers statistic, we used a modified Mantel's test and signed Bartlett's statistic

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to test for significant overall differences in the shape (Mantel's test) and size (Bartlett's statistic) of **G** in native and invasive populations (Goodnight and Schwartz 1997; Calsbeek and Goodnight 2009). We also tested for a significant difference in the rank of the two matrices. For G matrices, the rank is equal to n, the number of traits for which the genotypic variance is measured. However, it is possible for a population to have zero genotypic variance for one or more traits measured. In that case, the rank of the G matrix is n, the number of traits measured, minus the number of traits with zero genotypic variance. We performed a bootstrap analysis to assign statistical significance values to the selection skewers statistic, Mantel's test, Bartlett's statistic, and the difference in rank (see Calsbeek and Goodnight 2009 for complete bootstrap methods). We identified statistically significant differences in the G matrices by comparing the actual value generated by each test statistic to 1000 bootstrapped values.

To visualize the G matrix comparisons performed previously, we plotted three-dimensional ellipsoid representations of each G matrix estimated. The lengths of the axes of each ellipse are equal to the square root of the first, second, and third eigenvalue of **G** and represent the major axes of genotypic variance in each population. To ensure that all G matrices were comparable, we standardized each G matrix by a combined matrix that included the G matrices of the Czech Republic, France, Vermont and North Carolina (see full matrix standardization methods in Calsbeek and Goodnight 2009).

G matrix comparison: experimental estimation of selection differentials

One potential problem with comparing G matrices using the selection skewers method is that the hypothetical selection scenario chosen by the investigator may not reflect biologically realistic selection differentials. To address this problem, we repeated the selection skewers G matrix comparisons conducted previously using selection differentials for each population calculated during a selection experiment carried out in the same greenhouse as the aforementioned greenhouse experiment. A subset of genotypes from the greenhouse experiment (Czech, $N = 12$; France, $N = 6$; Vermont, $N = 9$; North Carolina $N = 9$) were grown in the greenhouse at two temperatures to represent both the current and the projected higher temperature that *Phalaris* may experience with climate change (current temperature: 21–24°C/15.5–18°C day/night; hot temperature: 31–34°C/21–24°C day/night). All plant preparation and experimental procedures followed (Lavergne and Molofsky 2007). A subset of the original 90 genotypes was used because of the space constraints of replicating 90 genotypes in eight different water and temperature treatments. The goal of this analysis was to determine whether invasive populations would be better able to respond to environmental changes because they have increased genetic variance relative to native populations (Lavergne and Molofsky 2007), or whether populations that have evolved under warmer conditions (more Southern latitudes) would, in general, be better able to respond to these selective pressures. At each temperature, we had four water treatments (25%, 50%, 100% and saturated). The 25% and 50% moisture treatments were determined by weight, and the 100% was kept well-watered, simulating an aerobic wet pasture or grassland environment. The saturated treatment was kept saturated by placing a 5-cm-tall saucer filled with water under each pot, simulating an anoxic wetland environment. There were two replicates per genotype per treatment for each temperature. For each experimental condition, the fitness of each genotype was recorded as the mean survival of the genotype replicates (0, 0.5, or 1) 10 weeks after initial planting. To determine which traits were under selection in each condition, we estimated fitness using viability selection estimates for all traits measured. Viability selection was measured using a nominal logistic regression of relative fitness on standardized trait means (Lande and Arnold 1983; Brodie et al. 1995) from the greenhouse experiment (Table 1). The traits included in each selection model were chosen using an AIC backward stepwise model (Burnham and Anderson 2002). Although we measured experimental selection gradients in eight greenhouse conditions, our goal was to use the estimated selection differentials from only one condition as our experimental selection scenario in the experimental selection skewers analysis. We chose to use selection differentials estimated from the hot 50% experimental condition for the experimental selection skewers comparison because this treatment produced the lowest survival and highest selection gradients of all experimental conditions (Table 1). The results of the AIC backward stepwise model for this scenario are shown in Table 2. For the two traits under selection, we calculated **G, P**, and the selection differential (s) for each population (Czech Republic, France, Vermont, and North Carolina) to use in the experimental selection skewers analysis (see Tables S5–S8). The selection differential (s) for each population was calculated by subtracting the mean value of each trait after selection (the mean values of emergence time and below-ground biomass as measured in the greenhouse experiment for all genotypes included in the selection experiment, weighted by survival) from the mean value of each trait before selection (the mean values of emergence time and below-ground biomass as measured in the greenhouse experiment for all genotypes included in the

Table 1. Selection gradients (ß), standard errors (SE), and *P*-values (*P*) estimated for selection in experimental greenhouse conditions consisting of two temperature regimes and four water treatments.

	% Water	Traits	ß	SE	*P*	% Survival
Cold	25	Below-ground mass	−0.176	0.068	0.0135	62.9
		Emergence time	−0.225	0.071	0.0049	
	50	Height	0.212	0.066	0.0043	48.6
		Tiller number	0.231	0.070	0.0033	
	100	Above Gr Dry Wt	0.093	0.041	0.0226	74.3
		Tiller number	−0.101	0.046	0.0819	
	Sat	Below-ground mass	−0.172	0.065	0.0209	65.2
		Emergence time	−0.249	0.067	0.0007	
Hot	25	*	*	*	*	8.6
	50	Below-ground biomass	−0.2338	0.108	0.0287	37.1
		Emergence time	−0.3378	0.114	0.0064	
	100	Tiller number	−0.0875	0.059	0.0146	67.1
		Emergence time	−0.1321	0.055	0.0344	
	Sat	Emergence time	−0.2008	0.079	0.0183	37.1

(*) Indicates that selection gradients on all traits were un-estimable because of low survival.

selection experiment). Again, we compared **G** between Northern native and invasive populations, Southern native and invasive populations, and between Northern and Southern populations within each range using the selection skewers method with selection differentials (s), **G**, and **P** derived from the selection experiment.

Table 2. Results of the backward selection model for hot 50% water greenhouse conditions. Models 3 and 4 are indistinguishable according to AICc. We used the traits in model 4 for the selection skewers analysis because all traits in the model are under significant selection.

Stepwise model	Traits	*P* (trait)	*P* (model)	AICc (model)
1	Below-ground biomass	0.0221	0.0557	373.74
	Emergence time	0.0199		
	Maximum tiller height	0.9017		
	Tiller number	0.8581		
	Above-ground biomass	0.1894		
2	Below-ground biomass	0.0186	0.0286	371.51
	Emergence time	0.0193		
	Tiller number	0.8909		
	Above-ground biomass	0.1269		
3	Below-ground biomass	0.0090	0.0126	369.33
	Emergence time	0.0189		
	Above-ground biomass	0.1220		
4	Below-ground biomass	0.0331	0.0145	369.64
	Emergence time	0.0037		

Results

G matrix comparison using hypothetical selection scenarios – native versus invasive populations

The first goal of our analysis was to determine whether the evolutionary trajectory of reed canary grass in the invasive North American range differed significantly from native European populations by comparing their responses to a hypothetical selection scenario. Overall, we found that correlations between the predicted responses to hypothetical selection scenarios were lower for comparisons of Northern and Southern populations within each range than between native and invasive populations (Table 3). These results indicate that there is a greater divergence in **G** along latitudinal gradients than between native and invasive populations. When comparing the G matrices of Northern native and invasive populations (Czech Republic vs. Vermont), we identified small differences in the size and shape of **G** (Table 3; Fig. 1A,C). The visual representations shown in Fig. 1 highlight minor differences in the genetic variance–covariance structure of the populations for emergence time, height, and tiller number, mostly encapsulated by an increase in genetic variance for emergence time in the invasive range, and a small change in the specific trait combinations that make the greatest contribution to genetic variance in the population (a change in the orientation of the major axis of genetic variance). These differences were identified by the change in rank statistic ($P = 0.02$), which contrasts the amount of genetic variance for each trait in the two populations, and Mantel's test ($P = 0.03$), which compares the overall shape of **G**. By contrast, Bartlett's test did not find any significant differences in the size ($P = 0.91$) of **G** in Czech Republic and Vermont, nor was there a

Table 3. Vector Correlations for the selection skewers G matrix comparisons of reed canary grass by range (native vs invasive), latitude (Northern vs Southern), and for individual populations by range and latitude for emergence time, height, and tiller number.

	Selection skewers		
Population comparison	Selection for decreased emergence time only	Selection for increased height only	Selection for increased tiller number only
Czech/Vermont	0.780	0.947	0.968
France/N. Carolina	0.702	0.977	0.986
Czech/France	0.273*	0.992	0.967
Vermont/N. Carolina	0.575	0.987	0.962

(*) Indicates marginally significant *P*-value ($P = 0.075$). The direction of selection applied in the selection skewers analysis is indicated by up (selection for increased trait values) or down (selection for decreased trait values).

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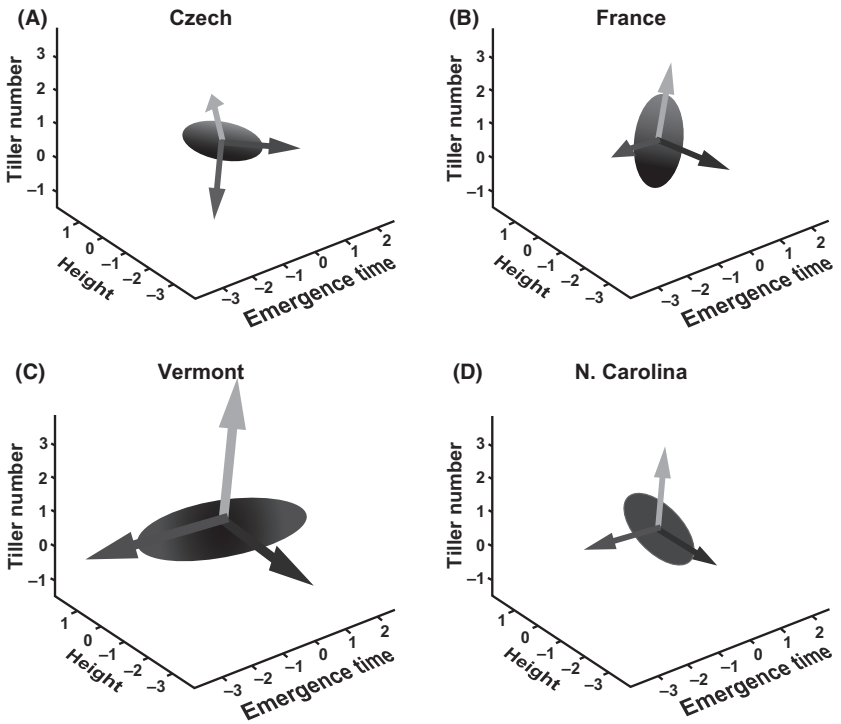

Figure 1 Ellipsoid representations of G for native and invasive populations of reed canary grass. The axes of the ellipsoid represent the major axes of genotypic variance, where the length of the axes of the ellipsoid are the square root of the first (λ_1), second (λ_2), and third (λ_3) eigenvalues of G, oriented in the direction of the first eigenvector. The G matrix for each population was measured for emergence time, height, and tiller number. For the Czech Republic, $\lambda_1 = 0$, $\lambda_2 = 1.06$, and $\lambda_3 = 2.11$, for France, $\lambda_1 = 0$, $\lambda_2 = 0.62$, and $\lambda_3 = 1.65$; for Vermont, $\lambda_1 = 6.85$, $\lambda_2 = 1.37$, and $\lambda_3 = 0.29$; and for North Carolina, $\lambda_1 = 0$, $\lambda_2 = 2.15$, and $\lambda_3 = 0.65$.

significant difference in the response to selection for decreased emergence time, increased height, or increased tiller number (Table 3).

We also compared **G** for native and invasive populations at the Southern margin of each range (France vs. North Carolina; Fig. 1B,D). Visually, the G matrices of France and North Carolina are similar in size and shape. In agreement with these results, we did not find any significant differences in the size (Bartlett's test; $P = 0.51$), shape (Mantel's test; $P = 0.66$), or rank ($P = 0.13$) of **G**, or any differences in the response to simulated selection on emergence time, height, or tiller number (Table 3).

G matrix comparison – latitudinal gradients within each Range

Finally, we compared the genetic architecture of reed canary grass populations along a latitudinal gradient within both the native and the invasive ranges to determine whether genetic architecture changes with latitude. In the native range, we compared the G matrices of Czech Republic and France, and in the invasive range, we compared **G** in Vermont to North Carolina. Visual comparisons of the G matrices in Czech Republic and France, and in Vermont and North Carolina, both highlight differences in the size and shape of **G** between Northern and Southern populations within each range (Fig. 1). However, we found no statistically significant difference in the rank ($P = 0.14$), size (Bartlett's test; $P = 0.86$) or shape (Mantel's test; $P = 0.98$) of the matrices for the Czech Republic and France. In addition, these populations would not experience significantly different responses to selection for increased height or tiller number (Table 3). We did detect a small, though nonsignificant ($P = 0.08$), difference in the expected response to selection for decreased emergence time in Czech Republic and France, suggesting that there may be minor differences in the genetic architecture of these populations (Table 3).

Within the invasive range, the G matrix comparison statistics indicated a significant difference in the rank of **G** in Vermont and North Carolina ($P = 0.02$): Vermont populations contained genetic variance for emergence time, whereas North Carolina populations have lost all genetic variance for this trait. However, no significant difference was found in the size (Bartlett's test; $P = 0.73$) or shape (Mantel's test; $P = 0.27$) of the two matrices, nor did we

detect any statistically significant differences in the expected responses to selection for decreased emergence time, increased height, or increased tiller number (Table 3).

G matrix comparison – experimental estimation of selection differential

The goal of the selection experiment was to estimate biologically relevant selection differentials (s) to use in place of the hypothetical selection scenario when comparing **G** using the selection skewers method. Table 4 lists the selection differential (s) calculated for each population during the selection experiment in hot, 50% water greenhouse conditions. We found multiple important similarities and differences in the estimated responses to experimental selection in *Phalaris* populations (Table 5). In agreement with the aforementioned selection skewers analysis using a hypothetical selection scenario, we found that the correlation between responses to experimental selection were lowest between Northern and Southern populations within each range, rather than between native and invasive populations (Table 5). Our results show very high correlations between the response to experimental selection in the native and invasive range comparisons (0.979 and 1.0; Table 5), meaning there would not be a significant difference in their responses to selection gradients induced by changing environmental conditions. By contrast, we found negative correlations between the responses to experimental selection in the Northern versus Southern population comparisons within each range (Table 5). Specifically, we found that there would be significantly different responses to experimental selection in Czech Republic versus France (Table 5). These results are also in agreement with the first selection skewers analysis, in that the majority of variation in the predicted responses to selection between populations was attributed to differences in latitude rather than between native and invasive ranges. Although we also found a negative correlation between the expected responses to experimental selection in Vermont and North Carolina, this difference was not statistically significant (Table 5).

Table 4. Selection differentials (s) calculated from the selection experiment.

	Emergence time (s)	Below-ground biomass (s)
Czech	−1.141	−0.603
France	−1.048	1.257
N. Carolina	−0.036	0.547
Vermont	0.515	−0.873

Table 5. Vector correlations between the predicted responses to experimental selection on emergence time and below-ground biomass by range (Native vs Invasive), latitude (North vs South), and within each range by latitude (Czech Republic vs France and Vermont vs. North Carolina).

Population comparison	Selection skewers statistic for selection on emergence time and below-ground biomass	*P*-value
Czech/Vermont	0.979	1.00
France/N. Carolina	1.00	1.00
Czech/France	−0.947	0.005*
Vermont/N. Carolina	−0.862	0.394

(*) Indicates *P*-value less than or equal to 0.05

Discussion

Recent studies have emphasized the need to explore how different evolutionary processes such as selection, migration, and drift interplay to influence the invasion success of introduced species into novel environments (Tsutsui et al. 2000; Lee 2002; Keller and Taylor 2008; Bacigalupe 2009). Specifically, simultaneous quantitative genetic analyses of native and invasive populations along environmental gradients are needed to (i) understand how founding events such as multiple introductions shape the evolutionary potential of introduced populations and (ii) determine how these changes either facilitate or constrain the adaptation and spread of introduced species along environmental gradients.

To address these challenges, we compared the genetic architecture of *Phalaris* populations in the native and invasive ranges, and along latitudinal gradients within each range. Overall, we found larger differences between the predicted responses to selection when comparing Northern and Southern populations within each range than between native and invasive populations. We did find a greater amount of genotypic variance in Vermont compared with the Czech Republic using the difference in rank statistic; however, we did not find any significant differences in the expected responses to either a hypothetical selection scenario or experimental selection in the native and invasive ranges (Tables 3 and 5). This implies that although the amount of genetic variance on which selection can act may be greater for some traits (i.e. emergence time) in invasive populations, the genetic constraints in the native populations have not been broken apart by the events of recombination that followed multiple introductions of European strains. Thus, invasive (Vermont and North Carolina combined) populations are not prone to evolve in new multivariate directions previously constrained in native (Czech Republic and France) populations.

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However, a very different picture emerges when latitudinal variation in genetic architecture is envisaged. When comparing Northern and Southern populations within each range, we found that genetic architecture varied significantly with latitude. We identified differences in the G matrices of Northern and Southern populations within each range that would cause differences in their evolutionary responses to similar selection pressures (Tables 3 and 5). Visual comparisons of **G** show differences in the overall shape and size of the G matrices in Northern and Southern populations within each range (Fig. 3), and there was a low correlation between their expected responses to a hypothetical selection scenario (Table 3). However, these differences (low correlations between expected responses to selection) were not always significant. This is likely due to the fact that the bootstrap algorithm used to calculate the test statistics is very conservative when used on G matrices estimated from small sample sizes, and the number of genotypes used in the individual population comparisons (Czech Republic $N = 28$; France $N = 13$; Vermont $N = 23$; North Carolina $N = 26$) was limited. However, the low correlations between the predicted responses to hypothetical and experimental selection scenarios (Tables 3 and 5) in Czech Republic versus France and Vermont versus North Carolina comparisons show that latitude is a confounding factor when comparing the genetic architecture of native and invasive populations. Taking into account the effects of latitudinal gradients while studying the evolution of invasiveness has been advocated but seldom tested rigorously (Keller and Taylor 2008; Colautti et al. 2009, 2010). In addition, these results suggest that variation in genetic architecture is driven by latitude and is not simply because of differences between the native and invasive ranges.

A previous study indicated that introduced Vermont and North Carolina *Phalaris* populations both resulted from multiple introductions of Czech Republic and France genotypes, with subsequent recombination (Lavergne and Molofsky 2007). This means that differences in **G** in Vermont and North Carolina are not simply the result of Northern native genotypes (from Czech Republic) establishing in Vermont, and Southern native genotypes (from France) establishing in North Carolina. Because of the history of multiple introductions in both introduced populations, the genetic architecture of these populations did not transfer unchanged from the native range. Thus, differences in **G** in Vermont and North Carolina are attributed to either strong genetic constraints imposed by the mix of genotypes that established in each population, or differences in selection occurring with latitude. A previous study showed that the overall genotypic variance in introduced populations is larger than their native counterparts because of multiple introductions (Lavergne and Molofsky 2007), and a likely scenario is that the increase in genotypic variance resulted in an increase in the evolutionary potential of introduced populations (Facon et al. 2005). This would have allowed for populations in Vermont and North Carolina to respond to local selection pressures and spread in their new environment. Selection can lead changes in G matrix structure over time (Jones et al. 2003). Thus, differences in selection by latitude in Vermont and North Carolina could explain the differences we observed in their genotypic covariance structure. Finally, in addition to the increase in genotypic variance in introduced *Phalaris* populations, differences in genetic architecture in Vermont and North Carolina also suggest that the invasion success of reed canary grass was facilitated by an increase in the evolutionary potential of introduced populations via the contribution of genetic variance from across the European range within each North American population, and subsequent recombination to create novel genotypes. Our overall results were supported by selection skewers G matrix comparisons using both hypothetical selection differentials and differentials calculated from experimental greenhouse conditions. These results suggest that the changes in **G** we identified between populations are biologically relevant, and our methods represent a novel approach to comparing the genetic architecture of native and invasive populations.

Together, our results show that the study of variation in genetic architecture between populations and along environmental gradients can give important insights into the evolutionary mechanisms driving range expansion. We showed that the overall genetic architecture and the potential response to selection have remained unchanged between the native and invasive ranges of reed canary grass when controlling for latitude. For the selection scenarios we tested, and the traits included in our analyses, we found that the main differences in the genetic architecture of reed canary grass populations occur between the Northern and Southern range margins. Thus, differences in the evolutionary potential of *Phalaris* populations, as represented by differences in **G**, are associated with differences in latitude. These results suggest a role for evolutionary processes such as adaptation in explaining the invasion success of introduced species and the need to account for geographic variation when tracing the introduction history and invasion potential of an introduced species (Colautti et al. 2009). More broadly, we have demonstrated the utility of using G matrix comparisons to investigate biologically relevant differences in the genetic architecture of invasive populations relative to their native counterparts and along latitudinal gradients within each range. Ideally, these methods will contribute to the future management of invasive species by allowing

us to determine the conditions under which these species pose the greatest threat of continued spread.

Data archiving

Raw data for this study are available in Dryad: doi: 10.5061/dryad.38d8k

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. G matrix for emergence time, height, and tiller number in Czech Republic.

Table S2. G matrix for emergence time, height, and tiller number in France.

Table S3. G matrix for emergence time, height, and tiller number in Vermont.

Table S4. G matrix for emergence time, height, and tiller number in North Carolina.

Table S5. G matrix for emergence time and below-ground biomass in Czech Republic.

Table S6. G matrix for emergence time and below-ground biomass in France.

Table S7. G matrix for emergence time and below-ground biomass in Vermont.

Table S8. G matrix for emergence time and below-ground biomass in North Carolina.

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