

Biogeography of a plant invasion: genetic variation and plasticity in latitudinal clines for traits related to herbivory

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Abstract. The juxtaposition of plant-species invasions with latitudinal gradients in herbivore pressure is an important yet mostly unexplored issue in invasion biology. Latitudinal clines in defense and palatability to herbivores are expected to exist in native plant species but the evolution of these clines may lag behind for invasive plant species resulting in non-parallel latitudinal clines that may impact invasion success. Our study focused on a native and European invasive lineages of the common reed *Phragmites australis* in North America. Using native and invasive genotypes of *P. australis* collected across a 17° latitudinal range, we performed experiments in replicate northern and southern common gardens to investigate whether these two lineages exhibited different genetically based latitudinal clines in defenses, nutritional condition, and palatability to their herbivores, the aphid *Hyalopterus pruni* and the fall armyworm *Spodoptera frugiperda*. We also tested whether invasive genotypes are more phenotypically plastic than native genotypes and whether plasticity varies with latitude. Although invasive genotypes did not exhibit higher defense levels (leaf toughness, phenolics, percent carbon), they were considerably less palatable to their herbivores than native genotypes. Genetically based latitudinal clines were evident for both native and invasive *P. australis* and for all defenses, nutrients, and at least one palatability trait for each herbivore. In 36% of the cases where clines were evident, they were non-parallel between the two lineages. These data suggest that clines in the invasive genotypes of *P. australis* evolved within the past ~100 years. Moreover, our study showed that the occurrence and direction of latitudinal clines in plant traits were commonly dependent on where the study was conducted (north or south), indicating strong phenotypic plasticity in these genetic-based clines. Finally, traits for invasive genotypes of *P. australis* were 2.5 times more plastic than traits for native genotypes. Interestingly, plasticity for native but not invasive genotypes was strongly dependent on latitude of origin. Such spatial heterogeneity within and between the native and invasive lineages of *P. australis* with respect to their interactions with herbivores can generate substantial spatial variability in biotic resistance that can have important implications for the establishment and spread of invasive genotypes and species.

Key words: biotic resistance; enemy-release hypothesis; *Hyalopterus pruni*; invasive species; latitudinal gradients; phenotypic plasticity; *Phragmites australis*; plant defense; plant–insect interactions; *Spodoptera frugiperda*.

INTRODUCTION

One of the most well-supported biogeographical patterns in ecology is the increase in primary productivity and species richness as latitude decreases (Rosenzweig 1995, Hillebrand 2004). Over the past several decades, much interest also has focused on latitudinal gradients in consumer–resource interactions (e.g., Coley and Aide 1991, Pennings et al. 2001, 2009, Schemske et al. 2009, Kim 2014, Cronin et al. 2015). For plant–herbivore interactions, herbivore damage is expected to increase toward

lower latitudes because of a longer growing season and more benign winter conditions (e.g., Dobzhansky 1950, Coley and Aide 1991, Bolser and Hay 1996, Pennings et al. 2001, 2009, Kozlov et al. 2015; but see Andrew and Hughes 2005, Moles et al. 2011). In response, natural selection should favor increased defenses or reduced palatability in plant species at lower than higher latitudes (Coley and Aide 1991, Schemske et al. 2009). Although this ecological paradigm is deemed too simplistic because it ignores feedbacks between plant defenses and herbivore abundance, and indirect and multi-trophic interactions (Kim 2014), latitudinal gradients in herbivory and defense are commonly reported. In the meta-analysis by Moles et al. (2011), 37% of the studies showed the expected negative relationship between latitude and herbivory. An additional 21% of the studies exhibited a positive relationship. Fifty-one percent of the studies

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exhibited a latitudinal gradient (positive and negative) in defense levels. Even though support for this paradigm is mixed, latitudinal gradients in herbivory, defense, and other traits related to plant–herbivore interactions are a common occurrence.

The invasion and subsequent spread of a nonnative species across a broad geographic range is expected to be followed by evolutionary changes in response to novel environmental and biotic gradients. A number of studies have documented that invasive species have rapidly evolved (<100 yr) in response to an environmental gradient in their introduced range. For example, invasive species have evolved distinct clines in growth and fitness-related traits with latitude (Maron et al. 2004, Novy et al. 2013, Li et al. 2015) that parallel the clines for the same species in their native range. Although the evolution of latitudinal clines in plant defenses or palatability to herbivores is expected to occur with invasive species, no study has ever examined whether or how quickly latitudinal clines have formed in traits related to plant–herbivore interactions.

The evolution of latitudinal clines in defenses and/or palatability traits in an invasive species may have important implications for invasion success (Bezemer et al. 2014, Cronin et al. 2015). For example, the biogeographic paradigm described previously predicts that native plants should exhibit latitudinal clines in defense and palatability traits (see, e.g., Pennings et al. 2001). However, an invasive species may not have had sufficient time to evolve a gradient that parallels the gradients for co-occurring native species. These non-parallel gradients in defense or palatability between native and invasive species, particularly early in the invasion process, may create large-scale heterogeneity in the relative impact of herbivores on co-occurring native and invasive plant taxa. As such, in some regions, herbivory may be greater on invasive than native plants (supporting the biotic resistance hypothesis; Levine et al. 2004, Chun et al. 2010) and in other regions, the reverse may occur (i.e., biotic susceptibility). In the only example on this subject, Cronin et al. (2015) found latitudinal gradients in herbivore pressure for native genotypes of *Phragmites australis* (Poaceae) in the field in North America. Invasive genotypes that are sympatric with native genotypes exhibited no latitudinal gradients in herbivore pressure. These non-parallel gradients in herbivory between native and invasive *P. australis* resulted in greater herbivore pressure on native than invasive genotypes in the south (supporting the local enemy-release hypothesis; Zheng et al. 2012) but no difference in herbivore pressure in the north. Importantly, the field study by Cronin et al. (2015) did not allow us to assess whether the geographic variation in *P. australis*–herbivore traits was genetically based, the result of plastic responses by the plants to an environmental gradient (e.g., climate), or some combination of the two.

Phenotypic plasticity, variability in the expression of traits in different environments, is another possible

mechanism that can generate spatial heterogeneity in native-invasive plant responses to herbivores. Phenotypic plasticity is thought to be beneficial to invaders because plastic “general-purpose genotypes” could have a fitness advantage in founder populations (e.g., Richards et al. 2006, Chun 2011, Davidson et al. 2011). Alternatively, plasticity may also evolve in an invasive species in response to the novel environment after colonization (Richards et al. 2006, Lavergne and Molofsky 2007). In their meta-analysis, Davidson et al. (2011) found strong support for the prediction that invasive species are more phenotypically plastic than native species. Moreover, theoretical models suggest that trait plasticity may vary along a climatic gradient, being greater at range margins than range interiors (Chevin and Lande 2011). A number of studies have demonstrated latitudinal gradients in trait plasticity (e.g., Maron et al. 2004, De Frenne et al. 2011, Woods et al. 2012). Consequently, we may expect co-occurring native and invasive species to differ in trait plasticity along a latitudinal gradient, which can contribute to spatial heterogeneity in invasion success. To date, no study has addressed this possibility.

Although the common reed, *Phragmites australis*, is native and widespread in North America, a continent-wide invasion by introduced European genotypes of *P. australis* in North America has been underway for at least 150 years (Saltonstall 2002). We conducted experiments in replicate common gardens, one in the north (41.49°, University of Rhode Island) and one in the south (30.35°, Louisiana State University), to assess whether genetic-based latitudinal gradients exist for different traits associated with the interactions between native and invasive *P. australis* and its herbivores. Because the study was conducted in a common-garden environment and maternal effects were minimized, any latitudinal patterns found would be genetically based. Using multiple native and invasive genotypes of *P. australis* collected across a 17° latitudinal range in North America (Fig. 1), we quantified plant defense levels (leaf toughness, total phenolics) and nutritional condition (percent carbon, percent nitrogen, C:N ratio, water content), and conducted experiments to assess palatability to two common and widespread generalist herbivores, the mealy plum aphid, *Hyalopterus pruni* (Homoptera: Aphididae), and the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). We tested the following six hypotheses: (1) Genetic-based latitudinal clines for plant defenses and palatability to herbivores are evident for native *P. australis* genotypes. (2) Because European invasive genotypes may not have had sufficient time to evolve in response to their novel environment, latitudinal clines in defense and palatability traits will be absent or weak, and thus non-parallel to the gradients for the native genotypes. Based on differences between replicate common gardens, we also tested the hypotheses that (3) invasive genotypes are more phenotypically plastic than native genotypes, (4) plasticity varies with latitude, and (5) latitudinal clines for each lineage differ between gardens,

indicating that these clines are phenotypically plastic. Finally, in light of our findings with regard to the previous hypotheses, we test the hypothesis that (6) across a broad latitudinal range in North America, European genotypes of *P. australis* are successful invaders because they are better defended and less palatable to herbivores than native genotypes. Support for this latter hypothesis would suggest that native communities have low biotic resistance to invasion by European *P. australis*.

METHODS

Study system

Phragmites australis is considered a model organism for the study of plant invasions (for a detailed discussion, see Meyerson et al. 2016). *Phragmites australis* is a tall and robust perennial grass of coastal and freshwater marshes with a nearly worldwide distribution (Marks et al. 1994, Lambertini et al. 2006). It was an uncommon species of wetland communities in North America for millennia but exhibited dramatic and rapid spread over the past ~150 years, particularly in the mid-Atlantic region of North America (Chambers et al. 1999). The rapid spread is attributed to the introduction of an invasive Eurasian genotype in mid 1800s (Chambers et al. 1999, Saltonstall 2002). Additional introduced genotypes have been discovered from the Atlantic and Gulf Coast regions of North America (Lambertini et al. 2012, Meyerson and Cronin 2013). At least 14 genotypes of native *P. australis* lineage are distributed throughout North America (Saltonstall 2002, Meadows and Saltonstall 2007) and have been reported to be threatened by the spread of invasive *P. australis* genotypes (Meyerson et al. 2010). Phylogenetic analysis revealed that native and introduced genotypes belong to two different clades (hereafter, lineages) often regarded as separate subspecies (Saltonstall 2002). Hybridization is shown to occur between native and invasive genotypes in nature (Saltonstall 2003, Paul et al. 2010, Saltonstall et al. 2014).

Among the most common and important herbivores of *P. australis* in North America are several introduced species, including the mealy plum aphid *H. pruni* and several species of gall flies in the genus *Lipara* (Diptera: Chloropidae) (Tewksbury et al. 2002, Lambert et al. 2007, Allen et al. 2015, Cronin et al. 2015). Native *P. australis* genotypes suffer substantially greater herbivore damage than the invasive genotypes (Lambert and Casagrande 2007, Allen et al. 2015, Cronin et al. 2015). Moreover, native genotypes but not invasive genotypes, exhibit latitudinal gradients in herbivore damage from the three major feeding guilds (sucking, stem-feeding [galling], and chewing) along the Atlantic and Gulf coasts of North America (Cronin et al. 2015). Leaf tissue loss from chewing herbivores and incidence of stem galls (primarily *Lipara* spp.) decreased with increasing latitude whereas densities of *H. pruni* increased with increasing latitude. These results suggest that native *P. australis*

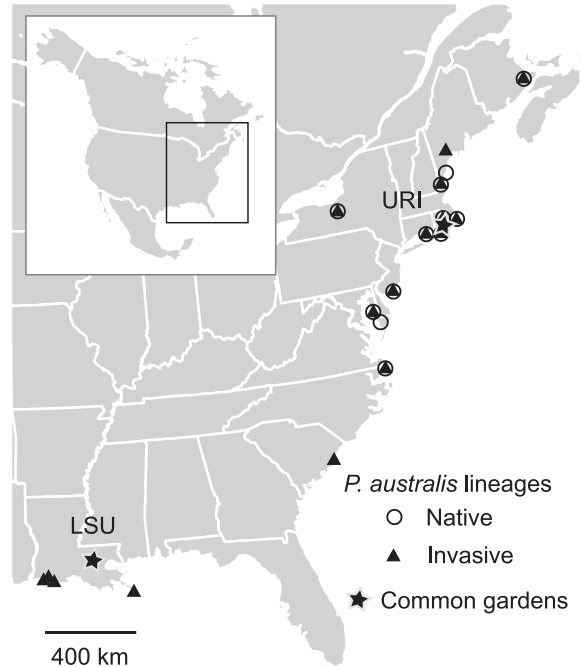


FIG. 1. Map of the location of *Phragmites australis* source genotypes used in this study and the location of the common gardens at Louisiana State University (LSU) and University of Rhode Island (URI), USA.

genotypes are more likely to have evolved latitudinal clines in response to herbivore pressure.

Plant defense, nutritional, and palatability traits in common gardens

We established replicate common gardens at Louisiana State University, Baton Rouge, Louisiana (LSU; 30.35° N, 91.14° W) and the University of Rhode Island, Kingston, Rhode Island (URI; 41.49° N, 71.54° W, Fig. 1). A small clump of rhizome was collected from 12 native and 16 invasive patches from the Gulf and Atlantic Coasts of North America (Fig. 1; Appendix S1). The rhizome material was split between the two gardens and propagated. Henceforth, we refer to each rhizome source as a source genotype. Owing to sprouting failures, mortality during propagation, and slow growth of some genotypes, the number of genotypes and their identity did not perfectly match between gardens at the time of our experimental studies. Seven native and 13 invasive genotypes survived at the LSU garden, whereas 10 native and 15 invasive genotypes survived at URI. Four native and eight invasive genotypes were common in both gardens in 2012 when the majority of the traits (defense, nutritional traits, and palatability to *H. pruni*; see *Palatability to H. pruni*) were quantified (Appendix S1). The URI garden represents a moderate temperate-zone climate, is roughly at the midpoint of the distribution of native genotypes used in our gardens, and is not far from where invasive *P. australis*

first became established in North America (Saltanstall 2002). In contrast, the LSU garden is subtropical, close to the southern range limit of invasive genotypes, and is ~700 km south of our southernmost native population.

We used identical methods for the propagation of plants in each garden, including soil type and watering, fertilization, and insecticidal treatment regimes. Rhizomes were planted in Metromix soil (Sun Gro Horticulture, Agawam, Massachusetts, USA) in 19-L nursery pots and maintained in outdoor plastic pools filled with fresh water. Plants were propagated vegetatively to get at least 32 pots per genotype. Therefore, all plants in both gardens that belong to a source genotype were clones. Source genotypes were randomly distributed within each garden. By growing the plants in the gardens for at least one year prior to the start of our study, maternal effects that might confound differences in plant traits were minimized. Plants were fertilized with Mega Green organic fertilizer (Hydrolysate Company of America, LLC, Isola, Mississippi, USA) upon detection of leaf yellowing and sprayed regularly with Safer insecticidal soap (Woodstream, Lititz, Pennsylvania, USA) to protect them from unwanted herbivores. Safer soap was used because it has a very short (<2 weeks) residual time on the plants. *Hyalopterus pruni* was the most common pest in the garden and the insecticidal treatment was effective in keeping them at low abundances leading up to the start of the experiments. All palatability experiments described in this paper were conducted with stems that had no visible herbivore damage or traces. We therefore expected little to no effects of background herbivores on plant growth and fitness traits.

Because we prevented seed production by clipping and removing panicles before seeds were produced and only rhizomatous growth occurred in our gardens, it was not possible for the clonal populations to evolve in response to the local climate. Consequently, differences in mean trait values between plants (clones) from the same source genotype, but from gardens separated by 11° latitude, should be the result of phenotypic plasticity in that trait (Maron et al. 2004, Colautti et al. 2009, Woods et al. 2012).

Caging experiments were performed to assess the palatability of *P. australis* genotypes to herbivores from two feeding guilds: the mealy plum aphid *H. pruni* and the fall armyworm *S. frugiperda*. *Hyalopterus pruni* is an invasive pest of dried plum (*Prunus domestica*) that was introduced from Europe and uses *P. australis* as a secondary host plant (Lozier et al. 2009). It is one of the most widespread herbivores of *P. australis* throughout North America and Europe and can produce massive outbreaks with severe damage to *P. australis* stands (Cronin et al. 2015). First recorded in California in 1881 (Smith 1936), it was probably introduced to North America after the introduction of invasive *P. australis* (Lozier et al. 2009). Consequently, the native and invasive lineages of *P. australis* in North America most likely had an equivalent amount of time to adapt to aphid herbivory and evolve clines. Therefore, we may expect parallel latitudinal gradients for traits associated with *H. pruni* herbivory between native and invasive *P. australis*.

Spodoptera frugiperda is native to North America and is a serious pest of cereal crops (Sparks 1979). It is known to feed on many grass species including *P. australis* (Sparks 1979, Bhattarai 2015). It overwinters only in the mild climates of the southern United States and reinvades most of the United States and southern Canada every year (Sparks 1979). The annual northward invasion by this species is likely to result into a latitudinal gradient in duration and intensity of herbivory on the host plants. Native genotypes of *P. australis*, which have had a potentially long history of interaction with these herbivores, are expected to exhibit latitudinal gradients for traits associated with the *P. australis*–*S. frugiperda* interaction. In contrast, such gradients may not exist for the invasive genotypes due to their relatively short history of interaction.

Because the phenological state of *P. australis* is likely to affect plant physiology, nutritional condition, defense levels, and herbivory (e.g., Liu et al. 2011, Lehndal and Agren 2015), our palatability experiments were designed to minimize differences in plant phenology between gardens and among source genotypes within gardens. However, phenology is a difficult concept to apply to grasses in the middle of their growing season because they are constantly producing new shoots. For example, within a pot of *P. australis* during late spring or summer, there is considerable variability in plant phenological state. Consequently, metrics such as growing degree days (GDD; McMaster and Wilhelm 1997) have limited value in these cases. Therefore, to minimize variation among experimental plants, we did the following. First, the experiments were timed to coincide with when the aphid colonies were well established and *S. frugiperda* caterpillars were present in the region. Second, we chose mid-size (growing) stems that were between 0.75 and 1.25 m in height. Finally, within a stem, we chose the uppermost, fully open leaf for *H. pruni* or the upper 5–10 leaves for *S. frugiperda* cages.

Palatability to H. pruni.—To assess the palatability of *P. australis* genotypes to aphids, we caged aphids on plants and measured colony growth. This bioassay (and other palatability metrics described here) integrates the effects of multiple plant defensive and nutritional traits on the herbivore's survival and reproduction (i.e., fitness) and is therefore a more holistic measure of plant resistance (Kim 2014). The source of aphids was a naturally occurring stand of *P. australis* within 80 km of each common garden. For ethical reasons and to minimize the genetic variation among aphids within each garden, we used a single source population of aphids for each garden. Cages for the aphid experiment were constructed using 5-cm lengths of clear acrylic tubing (2.8 cm in diameter, 0.1 cm in thickness) that were inserted over the *P. australis* leaves. The ends of the tubes were sealed with closed-cell foam plugs that had a small fine-screen window cut into their centers for air circulation (see Appendix S2: Fig. S1). Aphid colonies were initiated with two adult aphids caged

on the youngest fully open leaf on a randomly selected stem from each pot. Aphids reproduce parthenogenetically and produce a colony within a few days. After 10 d, leaves with aphid colonies were collected, transported on ice to the laboratory, and stored in a freezer at -20°C . With a suitable host, aphid colonies can increase in size by 100-fold in 10 d without any evidence of leaf deterioration or resource depletion due to intraspecific competition (G. P. Bhattarai, *personal observation*). Aphids per colony were enumerated and then dried at 40°C for 2 d. Dry mass of each colony was determined using a Mettler microbalance (0.1-mg precision) (Columbus, Ohio, USA). Because aphid mass was strongly correlated with aphid colony size ($R = 0.878$, $P < 0.0001$), we used only colony size in subsequent analyses. Aphid colony survival was determined as the proportion of cages per *P. australis* source genotype that had a viable aphid colony after 10 d. Survivorship and colony size were used as indicators of plant palatability to the aphids. The aphid experiment was conducted at LSU from 13 to 23 April 2012 with nine introduced and five native genotypes (20 cages per genotype), and repeated at URI from 17 to 27 June 2012 with 12 introduced and six native genotypes (8–12 cages per genotype). The experiments were performed two months later in the URI than LSU garden so that plants were in comparable developmental stages (about two months old) in each garden.

Palatability to S. frugiperda.—For *S. frugiperda*, we also performed a caging experiment and measured the performance of individual larvae feeding on plants from each source genotype. For both gardens, *S. frugiperda* were obtained as eggs from the same source (Benzon Research, Carlisle, Pennsylvania, USA) and larvae were reared in the laboratory on artificial diet provided by Southland Products (Lake Village, Arkansas, USA). At the fourth-instar stage, the mass of each larva was determined, and those larvae within 20–50 mg were selected for the experiment. Sleeve cages ($45 \times 60 \text{ cm}^2$ or $60 \times 75 \text{ cm}^2$ in size) were built using fine insect netting. The cage was inserted over the upper portion of a stem (containing 5–10 leaves) and enclosed around the stem at the bottom using a cable tie (see Appendix S2: Fig. S2). A single caterpillar was released into each cage through a hole cut open in the top of the cage. The hole was subsequently stapled closed. Within each pot, a single stem was selected at random for a cage. The experiment was terminated after 8 d, before any caged plants had all available leaf material consumed by the *S. frugiperda* and before the larva could mature to the pupal stage. Each larva was collected, transported on ice to the laboratory and its fresh mass was determined using a Mettler microbalance. Larval growth was calculated as proportional change in fresh biomass during the experiment ($\ln[\text{final mass}] - \ln[\text{initial mass}]$; mass measured in mg). Survivorship of larvae on each source genotype of *P. australis* was determined as the proportion of cages with a live larva at the end of the experiment. All the cases in which the larva died before

the termination of the trial were excluded from the analyses for palatability traits related to *S. frugiperda* (see *Methods: Statistical methods*).

We took photographs of all the remaining leaves inside the cage to quantify leaf area consumed by each larva. Using ImageJ (Rasband 2014), we quantified the remaining leaf area (cm^2) for each plant and estimated pre-consumption leaf area by extrapolation. Leaf area consumed by each larva was estimated as the pre-consumption leaf area minus leaf area remaining after 8 d. We used leaf area consumed and not proportion leaf area consumed because the total leaf area within a cage was variable and abundant (never being completely consumed), and therefore, the former measure is more informative. The amount of leaf area consumed by larvae could be an indicator of plant defense levels (e.g., Coley 1986). However, herbivores may also consume more to compensate for the lower quality of leaf tissues (Mattson 1980). Finally, we determined biomass conversion efficiency of larva (larval growth per \ln -transformed unit area of leaf consumed). Leaf area measurements for plants with dead or missing larvae were excluded from the analyses. These four variables, larval survivorship and growth, leaf area consumed, and biomass conversion efficiency, were used as the measurements of plant palatability to chewing herbivores. The experiment was performed on 23–31 May 2013 including nine native and 13 invasive genotypes (20 plants per genotype) at LSU garden, and on 7–15 August 2013, including six native and eight invasive genotypes (7–10 plants per genotype) at URI.

Plant defense and nutritional traits.—Plant characteristics related to defense and nutritional quality were measured concurrently with the *H. pruni* experiment and from the same pots as those with cages. Water content of leaves has been shown to have a positive relationship with population growth rate of aphids (e.g., Johnson 2008, but see Woods et al. 2012) and lepidopteran larvae (Scriber and Feeny 1979). Water content of leaves was estimated as the proportion of water per unit fresh biomass of three newly opened leaves collected from each pot ($n = 10$ per genotype). For nutrient analysis (percent carbon, percent nitrogen, and C:N ratio), the top three leaves were collected from a single plant per pot ($n = 5$ pots per genotype). Leaves were lyophilized and ground to a fine powder in the laboratory. Leaf nutritional levels were assayed using an elemental analyzer at Brown University Environmental Chemistry Facilities (equipment *described online*).⁵ Herbivores often prefer and perform better on plants with higher percent nitrogen (Mattson 1980, Agrawal 2004). Also, carbon content of leaves has been shown to influence herbivore performance (Agrawal 2004, Cronin et al. 2015).

Leaf toughness (force [measured in kg] required to push a blunt steel rod [4.8 mm in diameter] through the leaf) and total phenolics concentration were our measures

⁵ <http://www.brown.edu/Research/Evchem/facilities/>

of plant defenses against herbivores. As a member of the Poaceae, *P. australis* defenses are likely limited to structural defenses and phenolics (McNaughton 1979, Tschamtko and Greiler 1995, Strauss and Agrawal 1999). In our field surveys (Cronin et al. 2015), leaf toughness and phenolics were negatively related to leaf area consumed by chewing herbivores and aphid density, respectively. Using a penetrometer (Itin Scale Company, Brooklyn, New York, USA), toughness was measured for the fully open uppermost leaf from a randomly selected stem in each pot. Leaf toughness was also measured for the top-most leaf inside each cage of the *S. frugiperda* experiment in 2013. Total phenolics (nmol/g of dried leaf tissue) were estimated using a modified version of the Folin-Ciocalteu method (Waterman and Mole 1994, Cronin et al. 2015).

Statistical methods

Our primary objective with this study was to determine if *P. australis* trait variation within a common garden is genetically based, and particularly whether this genetic variation was attributed to differences between lineages (native, invasive) and/or was related to latitude of origin of the genotype (i.e., a genetic-based cline). A secondary objective involved using source genotypes that were shared between common gardens to assess whether traits associated with *P. australis*–herbivore interactions are more phenotypically plastic for invasive than native lineages. For the primary objective, we used mixed-effects or general linear models to test whether each plant defense, nutritional, and palatability trait varied between gardens, lineages, and along a latitudinal gradient. Garden (LSU, URI), lineage (native, invasive), and latitude of origin (hereafter, latitude effect) were treated as fixed effects, and source genotype was a random effect. With uniquely coded source genotypes (see Appendix S1), that belong to either native or invasive lineage, included in the model as a random effect our model structure was equivalent to a nested model (i.e., genotypes within a lineage).

Several traits required a slightly different analytical approach. Aphid colony size (number per cage) was Poisson distributed. Therefore, for this trait, we used a generalized mixed effect model with Laplace estimation method and Poisson distribution of errors (Bolker et al. 2009). To account for overdispersion of the count data, an observation-level random effect was also added to the model (Bolker et al. 2009). Because we had a single estimate of *H. pruni* and *S. frugiperda* larval survivorship per source genotype, we could not assess a source–genotype effect for these two traits. Therefore, survivorship of aphids and larvae were analyzed using general linear models. Finally, because we measured leaf toughness in 2012 and 2013, we included year as a random variable in the linear mixed-effects model for this defense trait.

To help normalize data distributions and homogenize variances among categories (i.e., gardens, lineages), percent

carbon, percent nitrogen, C:N ratio, total phenolics, and leaf area were ln-transformed. Quantile-quantile plots were also used to identify potential outliers in the distribution of trait estimates. However, in no case did the removal of these data points qualitatively change the conclusions of the model.

For each dependent variable, we used Akaike's information criterion corrected for finite sample size (AIC_c) to select the most informative model (Burnham and Anderson 2010). The full model included all random and fixed effects plus all two- and three-way interactions. In addition, we included a quadratic term (latitude^2) to evaluate whether the relationship between the trait and latitude was nonlinear. Candidate models were constructed using all possible combinations of the variables including one with no fixed effect, but with two restrictions. First, interaction terms could only be present in the model if their main effects were also present in the model. Second, the random effects (source genotype and the repeated measure of toughness) were retained in every model combination. Without this underlying structure to the model, the design would be pseudoreplicated.

Candidate models were ranked by AIC_c from lowest to highest value and AIC_c s with a Δ_i value ($AIC_{ci} - AIC_{cmin}$) of ≤ 2 were deemed to have substantial support (Burnham and Anderson 2010). We also report the AIC_c weights (w_i), which indicate the weight of evidence (as a proportion) in favor of model i being the best model given the set of candidate models. Goodness of fit of each mixed-effects model was reported as marginal ($R_{m^2}^2$, variance explained by fixed effects) and conditional R^2 (R_c^2 , variance explained by the entire model) that are comparable in interpretation to the coefficient of determination, R^2 , for linear models (Nakagawa and Schielzeth 2013). All analyses were run in R 3.2.0 (R Development Core Team 2015) using lme4 (Bates et al. 2014) and AICcmodavg (Mazerolle 2015) packages.

The AIC_c best models for each trait were used to evaluate the hypotheses outlined in the *Introduction*. The presence of a latitude effect in the model would suggest that there is a genetic-based latitudinal cline in *P. australis* (Hypothesis 1). The addition of a lineage \times latitude interaction in the best model would further indicate the existence of non-parallel latitudinal gradients between the native and invasive lineage (Hypothesis 2). Otherwise, the absence of this interaction term would suggest parallel latitudinal gradients (or, more appropriately, no evidence that gradients differ between lineages). A garden effect in the best model would suggest phenotypic plasticity in the trait (Hypothesis 3) and a garden \times latitude interaction would support Hypothesis 4 that the degree of plasticity for a trait (i.e., the difference between gardens) varies with latitude of origin of the plants. A three-way interaction between fixed effects (garden \times lineage \times latitude) would support our Hypothesis 5 that the slopes of the latitudinal clines for each lineage differs between gardens (i.e., the latitudinal gradient for each lineage is phenotypically plastic). Finally, if a lineage

effect is present in the best model and plant defense trait values are higher or palatability is lower for the native than invasive lineage, it would support the biotic-resistance hypothesis (Hypothesis 6).

In cases where the AIC_c best model included a garden interaction term (garden \times lineage, garden \times latitude, and/or garden \times lineage \times latitude interactions), we repeated the model-selection procedure for each garden to better elucidate the lineage and latitude effects on each trait. In this case, candidate models included all combinations of lineage, latitude, and the lineage \times latitude interaction, as well as a model with no fixed effects. If latitude (or latitude and lineage \times latitude interaction) was in the AIC_c best model for a particular garden, we performed mixed effect analyses to determine the relationship between latitude and the trait for each lineage.

We performed a separate correlation analysis for each garden to examine whether plant palatability traits were linearly related to defense and nutritional traits. Plant-level measures of putative defense (leaf toughness, total phenolics), nutritional (water content, percent nitrogen, percent carbon, C:N ratio) and palatability (aphid colony size, larval growth, leaf area chewed, and biomass conversion efficiency) were used in the pairwise correlation analysis (Pearson's product moment correlation, R). Nutrient concentrations and leaf area chewed were ln transformed. P values were adjusted for multiple comparisons using a Bonferroni correction.

For the second objective, we quantified phenotypic plasticity in defense, nutritional, and palatability traits for *P. australis* genotypes that were present in both common gardens (four native, eight invasive; Appendix S1). We could not assess plasticity in palatability to aphids because the aphids were obtained from different source populations located nearby each garden. As such, differences in palatability to aphids between gardens could also be due to genetic differences between aphid source populations (e.g., local co-adaptation between southern aphids and southern populations of *P. australis*). Plasticity for each trait and lineage was measured as the proportional difference in mean trait expression between gardens; e.g., effect size = [mean leaf toughness for native genotypes at LSU – mean leaf toughness for native genotypes at URI]/mean leaf toughness for native genotypes at LSU. Lineage means per garden for each trait were obtained from the least-squares means from the linear mixed-effects model outlined above (or the alternative models for larval survivorship, and leaf toughness) that contained garden and latitude as fixed effects and genotype as a random effect. For all traits, the effect size for native lineage (x -axis) was plotted against the effect size for invasive lineage (y -axis). If the data point for a trait falls above the 1:1 line, it would indicate that plasticity for that trait is greater for the invasive than native lineage. Data points below the line would indicate the opposite. A pairwise t test was performed to assess whether plasticity for the native and invasive lineages differed for all traits combined (Hypothesis 3). Finally, we

also computed plasticity for each source genotype of *P. australis* and used ANCOVA to examine the effects of lineage and latitude on plasticity for each defense and nutritional trait (Hypothesis 4).

RESULTS

Defense and nutritional traits

The phenotypic expression of *P. australis* defense and nutritional traits was strongly influenced by plant lineage and latitude of origin, and also was strongly modulated by where the study was conducted (LSU or URI). The best model, based on AIC_c weights, included latitude for all six traits (leaf toughness, total phenolics, water content, percent nitrogen, percent carbon, and C:N ratio) and lineage for four traits (total phenolics, water content, percent nitrogen, and C:N ratio; Table 1; Appendix S3). Interestingly, in all cases where lineage was present in the best model, so was a lineage \times latitude interaction; an indication that native and invasive lineages exhibit nonparallel genetically based gradients in these traits. Finally, not only was the garden where the study was conducted important in all cases (Table 1; see Appendix S3: Table S1 for detailed information), there were many interactions involving garden, lineage, and latitude of origin.

The defensive trait leaf toughness was negatively related to latitude, decreasing by 51% between our southernmost and northernmost genotypes (Fig. 2A, Table 1). Plants from the LSU garden exhibited steeper declines in toughness with increasing latitude than

TABLE 1. Best models, based on the corrected Akaike information criterion (AIC_c) model-selection procedure, for the effects of garden (G), lineage (S), and latitude (L) and all possible two- and three-way interactions on *P. australis* plant defense, nutrition, and palatability traits.

Dependent variable	Model
<i>Plant defense traits</i>	
Leaf toughness	G L G \times L
Phenolics	G S L G \times S G \times L S \times L G \times S \times L
<i>Plant palatability traits</i>	
Water content	G S L G \times S G \times L S \times L G \times S \times L
Percent nitrogen	G S L G \times S G \times L S \times L G \times S \times L
Percent carbon	G L G \times L
C:N	G S L G \times S G \times L S \times L G \times S \times L
Aphid colony size†	G S L G \times S
Aphid survivorship‡	G S L G \times S
Larval growth	G S
Larval survivorship‡	G S
Leaf area chewed	G S
Larval biomass conversion efficiency	G S L G \times S G \times L

Notes: Detailed information about these models is provided in Appendix S3: Table S1. Nutrient concentrations (percent carbon, percent nitrogen, C:N, and phenolics) and leaf area chewed (cm^2) were ln-transformed.

†Generalized linear mixed effect model (GLMM) with Poisson family was used in the analysis.

‡General linear models were used in these analyses.

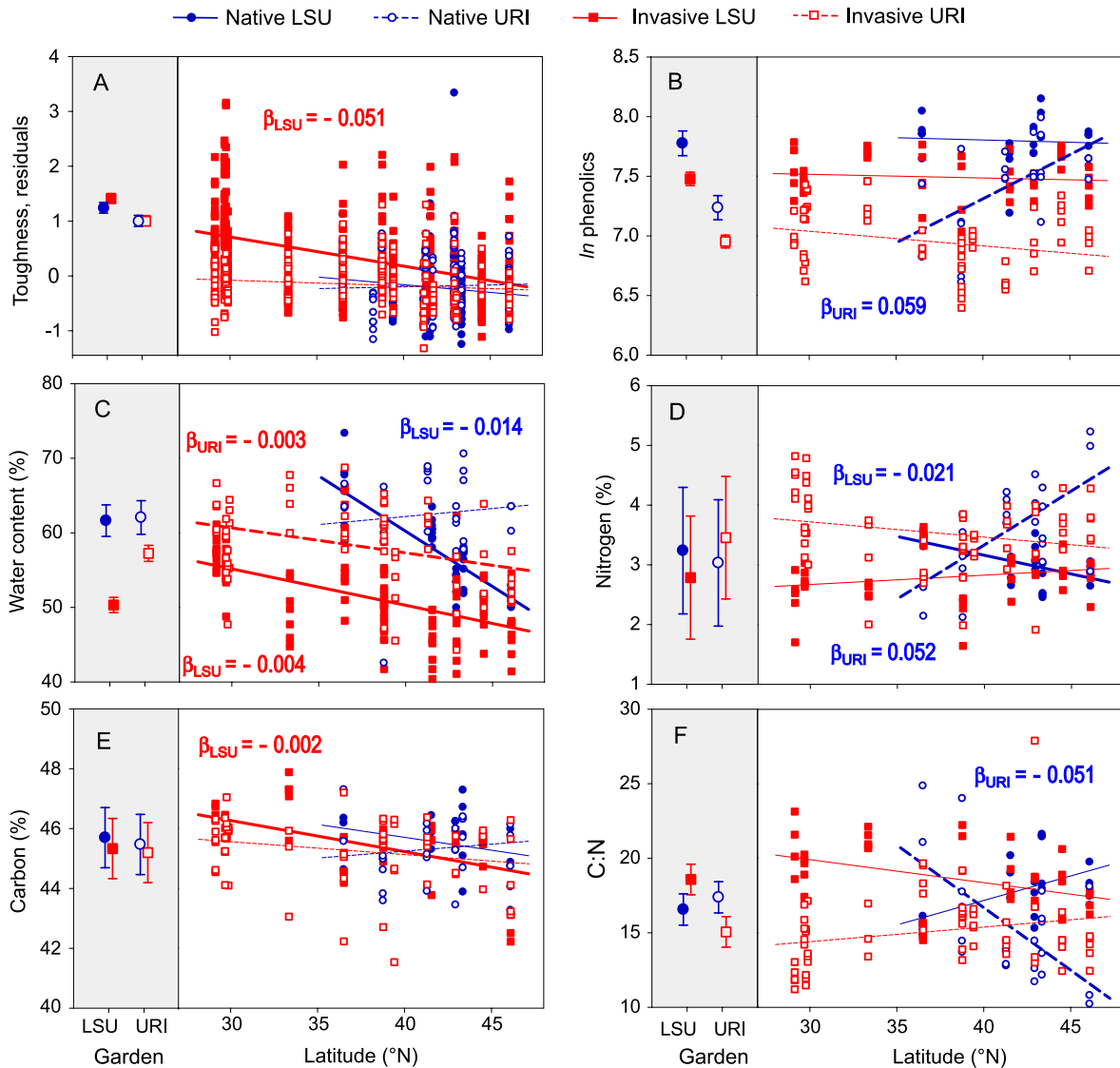


FIG. 2. Effects of lineage, latitude, and garden on defense and nutritional traits of native and invasive *P. australis* grown in common gardens at Louisiana State University and the University of Rhode Island. Symbols in the shaded portion of the graph are the least-squares means (\pm SE) for different lineages in different gardens. The relationship between a plant trait and latitude is shown by a line fit by least-squares regression, only for cases in which the AIC_c (corrected Akaike information criterion) best model included the effect of latitude (either latitude, lineage \times latitude, or garden \times latitude). Thick lines denote significant trait–latitude relationships ($P \leq 0.05$) and thin lines represent nonsignificant relationships ($P > 0.05$; see Appendix S3). Slopes (β_{LSU} and β_{URI}) are reported for the significant relationships. For leaf toughness [kg], we show the residuals from a linear mixed-effect model controlling for the effect of year.

plants from the URI garden (garden \times latitude interaction). Because of the garden \times latitude interaction in the best model, we conducted separate model-selection procedures for each garden to explore the effects of lineage, latitude and their interactions on leaf toughness. At the LSU garden, native and invasive lineages had parallel latitudinal clines in leaf toughness (no lineage \times latitude interaction in the AIC_c best model; Appendix S3: Table S2). There was a significant negative relationship between toughness and latitude for the invasive genotypes but the relationship was not

significant for the native genotypes (Fig. 2A; Appendix S3: Tables S2 and S3). In contrast, total phenolics were 34% higher for native than invasive genotypes and 96% higher for plants grown in the LSU vs. URI garden (Fig. 2B; Appendix S3: Tables S1, S4, and S5). At the LSU garden, total phenolics for invasive and native lineages did not vary with latitude of origin (Appendix S3: Table S2). However, at URI, total phenolics increased with latitude for native genotypes but did not vary with latitude for invasive genotypes (lineage \times latitude and non-parallel clines; Appendix S3: Tables S2 and S3).

Similar to total phenolics, the AIC_c best models to explain water content, percent nitrogen, and the C:N ratio were the same and included all main factors and their two- and three-way interactions (Table 1; Appendix S3). AIC_c weights for these models (the likelihood of the model given the candidate models considered) were >0.93. Water content was 8% higher for native than invasive genotypes and 4% higher for plants grown at URI vs. LSU (Fig. 2C; Appendix S3: Tables S4 and S5). At LSU, water content decreased with increasing latitude for both lineages but the latitudinal clines were non-parallel (lineage × latitude interaction in the AIC_c best model; Appendix S3: Tables S2 and S3). At URI, the invasive genotypes exhibited a significant negative latitudinal cline in water content but no cline was evident for the native genotypes. The absence of a lineage × latitude interaction in the AIC_c best models suggest that these two clines are not different (Appendix S3: Table S2). Lineage, latitude, and their interaction were important factors contributing to variation in percent nitrogen at URI but were unimportant to percent nitrogen at LSU (Fig. 2D; Appendix S3: Table S2). At the URI garden, percent nitrogen increased significantly with latitude for the native genotypes but declined (nonsignificant) with latitude for the invasive genotypes (non-parallel cline; Appendix S3: Table S3). Overall, percent nitrogen was similar between the native and invasive genotypes (3.13% ± 1.04% and 3.10% ± 1.02%, respectively; mean ± SE). For C:N ratio, the slopes of the latitudinal clines at URI were in the opposite direction as for percent nitrogen and was only significant for the native genotypes (Fig. 2F; Appendix S3: Tables S2 and S3). The difference in C:N ratio between lineages changed between gardens (garden × lineage interaction; Fig. 2F): the C:N ratio for invasive genotypes was 12.1% greater at LSU and 13.3% less at URI than for native genotypes. Finally, the AIC_c best model for percent carbon included only garden, latitude, and their interaction (Table 1; Appendix S3). Mean percent carbon was similar between native and invasive lineages (45.46% ± 1.01% and 45.25% ± 1.00%, respectively). On average, the percent carbon declined by 1.7% from the south to the north and the relationship was only evident for the plants at the LSU garden (hence the garden × latitude interaction; Fig. 2E; Appendix S3: Table S2). Only for the invasive lineage at LSU was there a significant latitudinal cline (Appendix S3: Table S3).

Palatability to herbivores

Traits associated with *P. australis* palatability to *H. pruni* and *S. frugiperda* were strongly influenced by the garden in which the study was conducted and plant lineage and, to a lesser extent, latitude. For aphid colony size, the best model included lineage, latitude, garden, and a garden × lineage interaction (AIC_c weight = 1.0; Appendix S3: Table S1). Aphid colony size was strongly affected by lineage and latitude at both gardens (both lineages; parallel negative latitudinal gradients; Fig. 3A;

Appendix S3: Tables S2 and S3). The garden × lineage interaction was present in the model because there was a 26-fold difference in colony size between native and invasive genotypes at LSU but only a fivefold difference at URI (Fig. 3A). After accounting for the garden effect, aphid colony size averaged 11 times larger for native than invasive genotypes (Appendix S3: Table S4). Based on paired measurements obtained from *P. australis* stems, aphid colony size was positively correlated with water content in both gardens (LSU $R = 0.39$, $P < 0.01$; URI $R = 0.23$, $P = 0.04$; Appendix S4) and total phenolics in the LSU garden ($R = 0.53$, $P < 0.01$; Appendix S4). No other nutritional or defense trait was correlated with this palatability measure.

Similar fixed effects were included in the best model for aphid survivorship (AIC_c weight = 0.51, $R^2 = 0.628$; Table 1; Appendix S3). Aphid survivorship was 32% higher on native than invasive plants and declined with increasing latitude (Fig. 3B; Appendix S3: Table S4). Mean survivorship was estimated to be 94% in the southernmost genotypes and 70% in the northernmost genotypes (a 24% change). As with colony size, the difference in aphid survivorship between native and invasive genotypes at LSU (46%) is much greater than the difference at URI (18%). At URI, the native and invasive lineages exhibited parallel negative latitudinal clines for aphid survivorship but at LSU, the negative latitudinal clines were not significant (Appendix S3: Tables S2 and S3). Because we had only a single estimate of aphid survivorship per *P. australis* source genotype, we could not assess correlations between this trait and nutritional and defense levels on a per-plant basis.

For the four traits associated with *P. australis* palatability to *S. frugiperda*, the main sources of variation in the AIC_c best models were garden and lineage (Appendix S3). For all traits, native genotypes were more palatable than invasive genotypes to *S. frugiperda* larvae. Larval growth rate, survivorship, leaf area consumed, and biomass conversion efficiency were 61%, 11%, 114%, and 33% higher on native than invasive genotypes, respectively (Fig. 3C–F; Appendix S3: Table S4). Interestingly, LSU plants were more palatable than URI plants. After accounting for the effects of lineage, larvae grew 142% larger, had 17% higher survivorship, consumed 65% more leaf tissue, and were 58% more efficient at converting plant biomass to larval biomass on LSU than URI plants (Fig. 3C–F; Appendix S3: Table S5). With regard to biomass conversion efficiency, the difference between lineages was only evident in the URI garden (garden × lineage interaction) and the relationship between conversion efficiency and latitude was affected by both lineage and garden (lineage × latitude and garden × latitude interactions). At the LSU garden, there was no latitudinal gradient in conversion efficiency (Fig. 3F; Appendix S3: Table S2). However, at the URI garden, conversion efficiency declined with increasing latitude but only for the invasive genotypes (but no lineage × latitude interaction; Fig. 3F; Appendix S3:

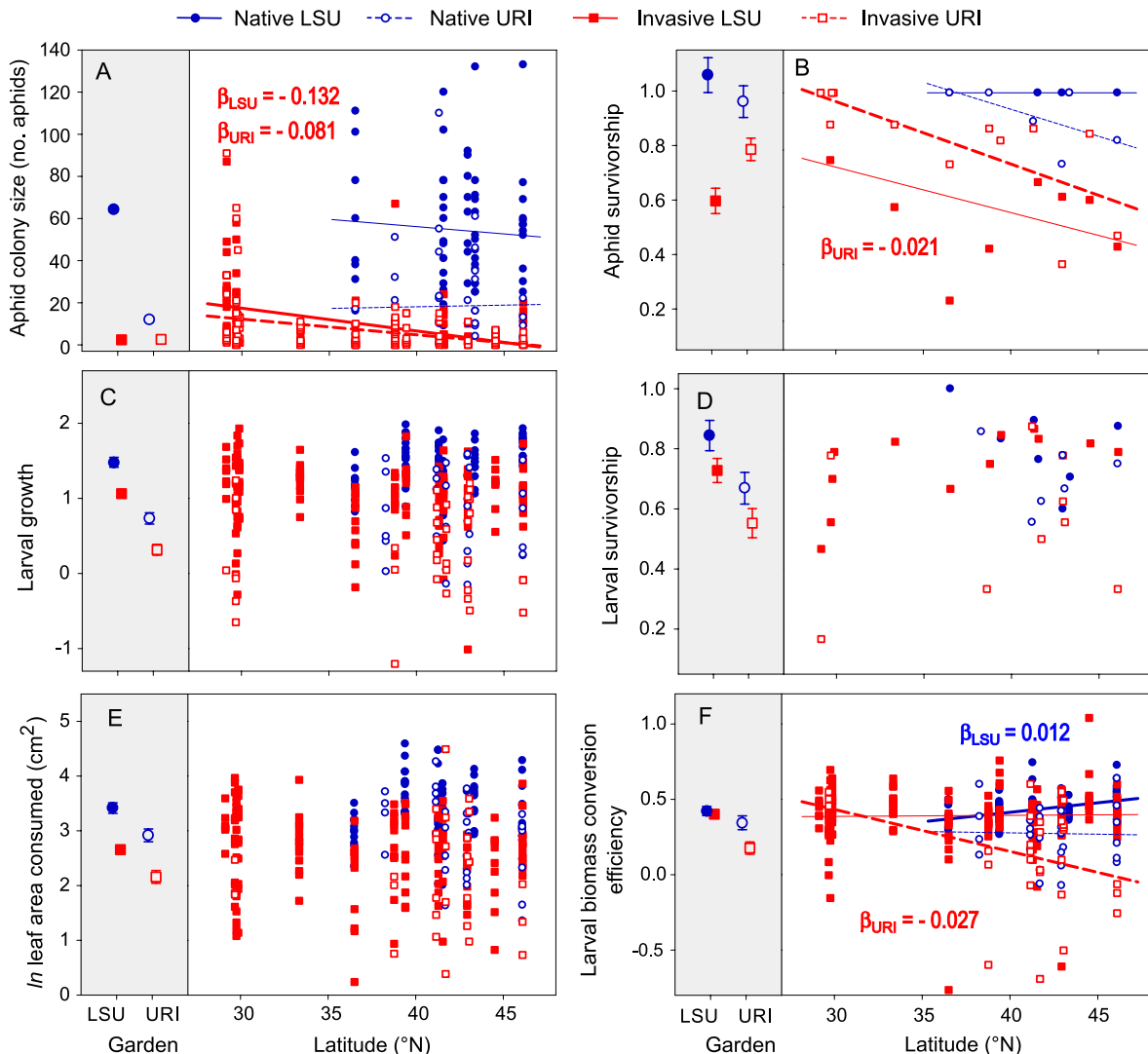


FIG. 3. Effects of lineage, latitude, and garden on palatability traits of native and invasive *P. australis* grown in common gardens at Louisiana State University and the University of Rhode Island. Symbols in the shaded portion of each graph are least-squares means (\pm SE) for native and invasive lineages in each garden. In cases where latitude, lineage \times latitude, or garden \times latitude was a factor in the AIC_C best model, the relationship between the plant trait and latitude is shown by a line fit by least-squares regression. Thick lines denote significant trait–latitude relationships ($P \leq 0.05$) and thin lines represent nonsignificant relationships ($P > 0.05$; see Appendix S3). Slopes (β_{LSU} and β_{URI}) are reported for the significant relationships. Survivorship on each source genotype of *P. australis* was determined as the proportion of cages with a live larva or aphids at the end of the experiment; larval growth was calculated as proportional change in fresh biomass during the experiment ($\ln[\text{final mass}] - \ln[\text{initial mass}]$); mass measured in mg; leaf area consumed was measured in square centimeters; biomass conversion efficiency of larva was measured as larval growth per \ln -transformed unit area of leaf consumed.

Tables S2 and S3). Finally, *S. frugiperda* palatability was generally negatively correlated with leaf toughness and positively correlated with percent nitrogen (Appendix S4). The correlations were strongest in the LSU garden where leaf toughness was significantly negatively related to larval growth rate ($R = -0.21$, $P < 0.01$) and leaf area chewed ($R = -0.20$, $P < 0.01$) and nitrogen was significantly positively correlated with biomass conversion efficiency ($R = 0.40$, $P = 0.04$). At URI, the direction of the relationships between leaf toughness and percent nitrogen and *S. frugiperda* palatability were similar to those found

in the LSU garden but only one relationship was significant: larval growth rate and percent nitrogen ($R = 0.60$, $P = 0.05$). Interestingly, water content was negatively correlated with leaf-area chewed in the LSU garden ($R = -0.26$, $P = 0.04$).

Plasticity in trait responses

We found strong evidence that plant traits associated with herbivory in *P. australis* were phenotypically plastic, more so for invasive than for native genotypes. Averaged

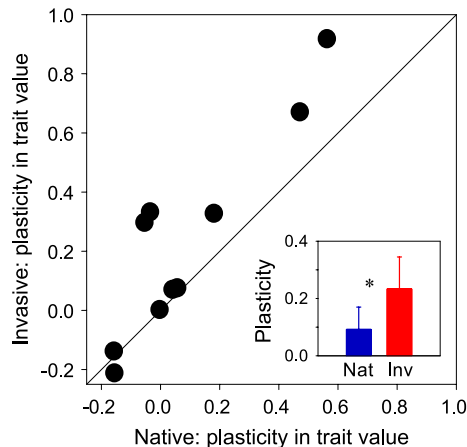


FIG. 4. Phenotypic plasticity in defense and palatability traits for native and invasive genotypes of *P. australis*. Plasticity in plant traits ($n = 10$) was estimated as the proportional difference in mean trait expression between gardens (i.e., [mean trait value for a lineage at LSU – mean trait value for that lineage at URI] / mean trait value for LSU). Symbols above the 1:1 line depict greater plasticity in invasive genotypes than the natives. Inset bar graph shows least squares (mean + SE) plasticity for native (Nat) and invasive (Inv) genotypes. A statistically significant difference between means (pairwise t test; $P = 0.024$) is represented by an asterisk.

across all 10 traits (aphid traits were excluded; see *Methods*), plasticity (proportional difference between the two gardens; mean \pm SE) for the invasive genotypes was 0.23 ± 0.11 and for the native was 0.09 ± 0.08 ; a statistically significant 156% difference ($t_9 = 2.708$, $P = 0.024$, Fig. 4). These large differences in trait expression between gardens, for both lineages, likely underlie the ubiquitous garden effect in the previous analyses (Appendix S3: Table S1).

Irrespective of *P. australis* lineage, plants reared at the URI garden were more nutritious and less well defended than those at LSU. Plants reared at URI had 7% greater leaf water content and had 24% more nitrogen than those at LSU, whereas plants at LSU produced 131% tougher leaves, 58% more total phenolics and 23% greater CN ratio than plants at URI (Appendix S5). Despite the higher defenses and lower nutrition at LSU, plants grown at LSU were more palatable to herbivores than those at URI. Aphid colony size was 57% greater at LSU than at URI (Appendix S5). *S. frugiperda* larvae consumed two times more leaf area and exhibited a 244% higher growth rate at LSU than in URI (Appendix S5). Finally, biomass conversion efficiency of larvae was 137% greater at LSU than URI (Appendix S5).

When trait plasticity was measured for each *P. australis* genotype, we found that it varied with latitude for four of six traits in native genotypes and only one of six traits in invasive genotypes (Fig. 5; Appendix S6). For native genotypes, plasticity in leaf toughness, water content, and percent nitrogen decreased (Fig. 5A,C,D) and plasticity in the C:N ratio increased (Fig. 5F) with

increasing latitude of origin. For the invasive genotypes, leaf toughness was the only trait in which plasticity varied with latitude, a relationship that paralleled the one for native genotypes (Fig. 5A). The difference between lineages in the relationship between plasticity and latitude for total phenolics, water content, percent nitrogen, and C:N ratio are indicated by a significant lineage \times latitude interaction in the model ANCOVAs (Appendix S6). We also examined whether these latitudinal trends were driven by invasive genotypes collected from southern latitudes beyond the distributional range of native genotypes. Reanalyses of these data excluding those genotypes (collected from the sites south of 36° latitude) did not qualitatively alter the results.

DISCUSSION

The juxtaposition of plant species invasions with large-scale gradients in herbivore pressure and native plant–herbivore interactions is an important yet mostly unexplored issue in the field of invasion biology (Bezemer et al. 2014, Cronin et al. 2015). Our study is the first to demonstrate genetic-based latitudinal clines for traits related to plant–herbivore interactions involving sympatric invasive and native species (or lineages of the same species). These data suggest that clines in the invasive genotypes of *P. australis* evolved within the past ~ 100 years. In 36% of the cases where clines were evident, the clines for native and invasive lineages were not parallel. Moreover, our study showed that the occurrence and direction of latitudinal clines in plant traits was commonly dependent on where the study was conducted (LSU or URI), strongly suggesting that environmental context is an important driver of the expression of plant traits and clines in those traits. Until now, this phenotypic plasticity in latitudinal clines for traits related to species interactions has never been reported. Finally, we found that invasive genotypes were 2.5 times more plastic than native genotypes but the native genotypes were much more likely to exhibit significant latitudinal variability in phenotypic plasticity than the invasive genotypes. Overall, this study suggests that traits associated with *P. australis* interactions with its herbivores are under strong genetic and environmental controls and they vary between co-occurring native and invasive *P. australis* genotypes across their latitudinal range in eastern North America. Such spatial heterogeneity within and between lineages with respect to their interactions with herbivores has the potential to generate substantial spatial heterogeneity in biotic resistance/susceptibility that can have important implications for the establishment and spread of invasive genotypes and species.

Latitudinal clines in plant–herbivore traits

*Hypothesis 1: Genetic-based latitudinal clines for plant defenses and palatability to herbivores are evident for native *P. australis* genotypes.*

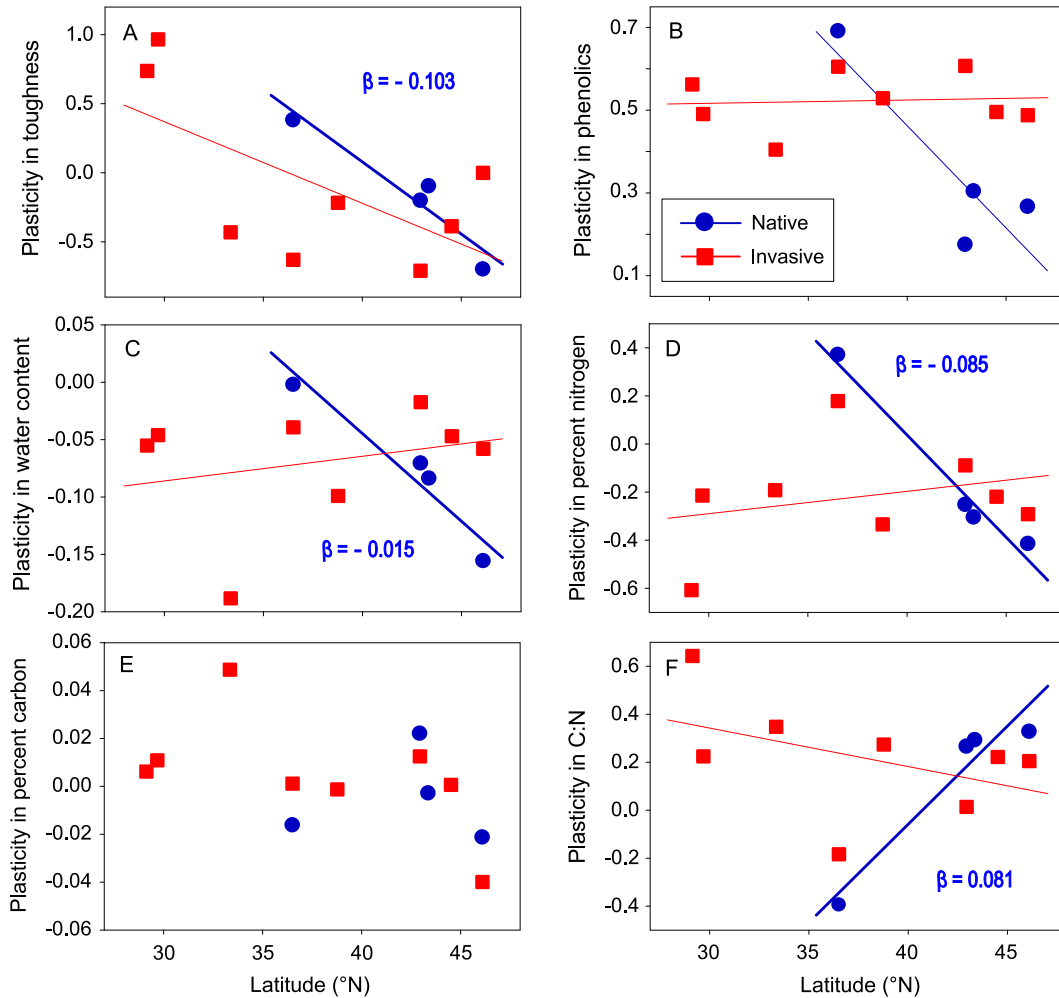


FIG. 5. The relationship between trait plasticity (proportional difference in mean trait expression between gardens) and latitude for native and invasive *P. australis* genotypes. Each point represents a single genotype that was represented in each garden. Lines are fit by least-squares regression and were provided only for those traits in which a significant latitude or lineage \times latitude interaction was detected in an ANCOVA (see Appendix S6). Slopes (β) are reported for the significant relationships.

Hypothesis 2: Because invasive genotypes may not have had sufficient time to evolve in response to its novel environment, latitudinal clines in defense and palatability traits will be absent or weak, and thus non-parallel to the gradients for the native genotypes.

Genetic-based clines.—In our study, support for the hypotheses that native genotypes should be more likely to exhibit genetic-based latitudinal clines than invasive genotypes (Hypotheses 1 and 2) was mixed. At least one cline was evident for each defense and nutritional trait, and for at least one trait associated with palatability to *H. pruni* and *S. frugiperda*. However, between the two gardens, there were twice as many latitudinal clines in plant–herbivore traits for the invasive genotypes compared to the native genotypes (eight vs. four clines, respectively; Appendix S3: Table S3). Moreover, in only one instance was a latitudinal

cline for a particular trait evident for both lineages (water content).

Although it was strongest in the LSU garden, leaf toughness of native and invasive genotypes generally increased with decreasing latitude. As herbivore pressure in naturally occurring patches of *P. australis* is higher at lower latitudes (Cronin et al. 2015), our common-garden study supports the presumed role of this trait as a defense against herbivores (e.g., Raupp 1985, Salgado and Pennings 2005). Its role as a defense is further supported by the finding that palatability to *S. frugiperda* was generally negatively correlated with leaf toughness. Our results with leaf toughness are also consistent with the prediction that longer lifespans of leaves in areas with longer growing seasons should favor tougher leaves (Coley and Aide 1991, Salgado and Pennings 2005). The lone positive latitudinal cline for total phenolics, evident for native genotypes at URI, was the opposite of what we

observed in the field (Cronin et al. 2015) and is counter to our expectations if this trait is related to herbivore defense. However, total phenolics represent a broad class of compounds that serve other purposes for plants including protection against photodamage (Close and McArthur 2002). Moles et al. (2011) reported that ~30% of the published studies found a significant latitudinal gradient in plant total phenolics. Interestingly, the majority of those significant gradients were in the direction observed for the native genotypes from the URI garden. Finally, these results are also consistent with the findings from the meta-analysis of common-garden studies by Colautti et al. (2009) in which there was no consistent directionality in latitudinal clines for plant defense traits, and that evidence for clines in the field do not always match up with clines for the same species in the common garden.

Latitudinal gradients in foliar nutrient levels are commonly reported (e.g., Siska et al. 2002, Reich and Oleksyn 2004, Lovelock et al. 2007, De Frenne et al. 2013, He and Silliman 2015). Between gardens, there was a trend toward decreasing percent carbon with increasing latitude, the opposite pattern to that observed in nature (e.g., De Frenne et al. 2013). It is possible that plants adapted to the CO₂-rich environments in the north may have evolved to be less efficient at uptaking or utilizing CO₂ for photosynthesis or storing carbon compounds into their tissues than plants from the relatively CO₂-poor environments in the south (see, e.g., Denning et al. 1995). For example, plant stomata are known to stay open longer and wider or the density of stomata increase when the concentration of atmospheric CO₂ decreases in order to maintain an adequate CO₂ gradient between the atmosphere and the leaf (Beerling et al. 1998). It is possible that *P. australis* adapted to their local CO₂ concentrations and consequently, southern plants were more efficient at producing or storing carbon compounds. Alternatively, carbon-rich tissues at southern latitudes could serve as a defense against greater herbivore pressure in the south (Orians and Milewski 2007).

We found evidence for latitudinal clines for percent nitrogen, C:N ratio, and water content but they varied between gardens and lineages. Although nitrogen content in coastal wetland plants tends to increase with increasing latitude (Siska et al. 2002, He and Silliman 2015), we found both positive (URI garden) and negative (LSU garden) latitudinal clines for percent nitrogen in native *P. australis* genotypes. Similar results were found for the C:N ratio and water content. These differences between lineages and gardens clearly indicate that latitudinal clines in *P. australis* traits are phenotypically plastic (Woods et al. 2012; *Phenotypic plasticity in defense and palatability traits*).

The genetic-based clines for palatability to *H. pruni* (aphid colony growth and survivorship decreased with increasing latitude; although more strongly for the invasive lineage) do not appear to be caused by latitudinal variation in the *P. australis* nutritional and defensive

traits. Although aphid colony growth was positively correlated with total phenolics and water content of leaves, neither of those variables was positively correlated with latitude. Latitudinal gradients in palatability to aphids could be explained by the fact that aphid abundance on *P. australis* increases with increasing latitude in North America (Cronin et al. 2015). At northern latitudes, strong selection pressure by these herbivores may have resulted in higher resistance or lower palatability.

Because *H. pruni* was introduced to North America not long after the introduction of invasive genotypes of *P. australis* (Lozier et al. 2009), both native and invasive lineages likely had an equivalent amount of time to adapt to the gradient in aphid abundance. The existence of parallel clines between native and invasive genotypes suggests that both *P. australis* lineages evolved in similar ways to the latitudinal variation in aphid abundance. A few studies to date have reported the formation of clines along an environmental gradient (latitudinal or elevational) by an invasive species that parallels the clines in the native range (Maron et al. 2004, Alexander et al. 2009). However, most previous studies (e.g., Maron et al. 2004, Colautti et al. 2009) involved cross-continental comparisons between native and invasive species and so, environmental conditions, even at similar latitudes, may not be the same. In contrast, our native and invasive genotypes are found in the same marshes along the East and Gulf Coasts and experience the same environmental gradient.

With only one exception (negative correlation between biomass conversion efficiency and latitude for the invasive genotypes in the URI garden), there was very little evidence of genetic-based clines for palatability to *S. frugiperda*. In nature, chewing damage to native genotypes of *P. australis* is strongly negatively correlated with latitude (Cronin et al. 2015). In response to these selection pressures, we expected that the native genotypes would have evolved a positive latitudinal cline in palatability to native grass-feeding generalists like *S. frugiperda*. The fact that a gradient was evident for the invasive genotypes, and that it was in the opposite direction predicted for the native genotypes is surprising. Finally, we suggest that the negative relationship between leaf toughness and latitude (Fig. 2) could be the mechanism driving the negative latitudinal gradient in biomass conversion efficiency in the invasive lineage.

Non-parallel latitudinal gradients between native and invasive genotypes.—One interesting and important finding regarding native and invasive genotypes of *P. australis* was that for 20% of the cases (5 of 24; 12 traits × 2 gardens), the relationship between latitude and trait level was different for native and invasive genotypes from the same garden (in support of Hypothesis 2). We observed non-parallel clines in water content, total phenolics, percent nitrogen, and C:N ratio (all but the first occurring in the URI garden). In the case of total phenolics, percent nitrogen, and C:N ratio, the slope of the relationship with

latitude differed in sign between the native and invasive genotypes. These results suggest that the two lineages have evolved in different ways to the same environmental gradient. Although there are many studies that have examined latitudinal clines for species in their native and invasive ranges (Colautti et al. 2009), environmental differences make clinal comparisons questionable. Because the native and invasive ranges of *P. australis* overlap on the same continent, our study provides a much stronger example of clinal evolution in invasive taxa. Although co-occurring native and invasive genotypes across a broad latitudinal range is known only for *P. australis*, a number of other species have co-occurring and distinct invasive genotypes or native-invasive hybrids (Ayres et al. 2004, Lavergne and Molofsky 2007, Ciotir et al. 2013). These are potentially fertile systems for the study of cline formation and evolution. These non-parallel latitudinal clines between co-occurring native and invasive genotypes of *P. australis* can result in spatially varying degrees of local enemy release and biotic resistance (see *Implications for invasion success*).

Origin of latitudinal clines.—We contend that the clines described in this paper for the invasive genotypes of *P. australis* must have arisen *de novo* while in North America. A preexisting cline, i.e., one that evolved in Europe and subsequently transferred virtually intact to North America, is possible but unlikely. For this to occur, there would have to have been multiple introduction events in which individuals from one latitude in Europe colonized at a similar latitude in North America. There is no evidence for this sort of parallel invasion process with *P. australis*. Based on an analysis of chloroplast DNA from herbarium specimens by Saltonstall (2002), European *P. australis* became established in the mid-Atlantic region at least 150 years ago and spread rapidly north and south (Saltonstall 2002). It is highly suggestive of one or a few major founding events followed by range expansion. Although other European genotypes are present in North America, they appear restricted to very northern and southern latitudes (Lambertini et al. 2012, Meyerson and Cronin 2013). Therefore, our study supports the growing body of literature that invasive species evolve relatively quickly (<100 yr) in response to an environmental gradient (e.g., Maron et al. 2004, Alexander et al. 2009, Li et al. 2015). Furthermore, the absence of a humped or u-shaped relationship between latitude and each trait, centering on the origin of the invasion (between Delaware and Connecticut; Saltonstall 2002), suggests that the time since invasion is not an important driver of the evolution of these gradients in the invasive lineage.

Our finding that invasive genotypes were twice as likely as native genotypes to exhibit a latitudinal cline for plant-herbivore traits is somewhat surprising. Such a result would suggest that the invasive genotypes in their new range were more evolutionarily responsive to the same environmental conditions faced by the native genotypes. For two main reasons, this seems unlikely. First, local

adaptation and cline formation requires limited gene flow among populations distributed along the latitudinal gradient (Slatkin 1985). Populations of native genotypes are quite rare and isolated in comparison to the extremely widespread and abundant invasive populations (Chambers et al. 1999, Saltonstall 2002). The native genotypes would seem much more likely to exhibit local adaptation and genetic isolation-by-distance than invasive genotypes. This prediction is supported by Kettenring and Mock (2012) who found greater genetic homogeneity among invasive than native populations of *P. australis*. Second, the selection pressures from North American herbivores on the invasive genotypes is considerably weaker than on native genotypes. Based on our latitudinal field surveys of herbivory, invasive genotypes suffered 70–650% lower levels of herbivory from all herbivore guilds (chewers, gallers, suckers) than native genotypes and that herbivory levels for invasive genotypes did not vary significantly with latitude (Cronin et al. 2015). Moreover, this study demonstrated that two herbivores, *H. pruni* and *S. frugiperda*, had lower growth and survivorship on the invasive genotypes. If anything, the high levels and strong latitudinal gradients in herbivory of the native genotypes should have favored a high frequency of genetic-based latitudinal clines for this lineage.

Trait variation between replicate common gardens.—One of our strongest and most obvious findings was that for all traits considered in this study, a garden effect was present in every model; indicative of significant trait plasticity. Also, interactions involving garden (e.g., garden \times lineage, garden \times latitude, garden \times lineage \times latitude) were quite common (see Appendix S3). These types of garden effects are a frequent occurrence in studies involving multiple common gardens (e.g., De Frenne et al. 2012, Woods et al. 2012, Zhou et al. 2014). Also, in their meta-analysis, Colautti et al. (2009) found numerous examples of a garden \times latitude interaction for studies conducted in both the native and invasive ranges of a plant species. In some studies, reversals of latitudinal trends were evident between gardens (Chapin and Chapin 1981, Santamaría et al. 2003, Maron et al. 2004). As the same species or genotype is represented in each garden, these garden \times latitude interactions imply plasticity in the regulation of latitudinal clines (Richards et al. 2006, Woods et al. 2012, Zhou et al. 2014).

One possible reason for the substantial differences in trait expression between gardens is that the gardens reside in very different parts of the range of the two *P. australis* lineages. The URI garden is near the center of the coastal range for both native and invasive lineages, whereas the LSU garden is near the southern extent of the invasive lineage and ~700 km south of our southernmost native population. Plants in the southern garden, particularly for the native lineage, may be at their thermal limits (e.g., Drake et al. 2015), which could result in altered expression of genetic-based clines in these plant nutritional traits. Several studies have examined latitudinal

clines in plant traits in replicate common-garden experiments located within, at the boundary or beyond the limits of the species range (e.g., De Frenne et al. 2012, Woods et al. 2012, Zhou et al. 2014). For example, in their study of plant growth and defense traits in common milkweed (*Asclepias syriaca*), Woods et al. (2012) found a cline in milkweed phenology in their garden at the southern range limit (North Carolina) but no cline in their garden at the northern range limit (New Brunswick, Canada). Woods et al. (2012) also evaluated whether plant defense trait expression was dependent on the proximity of the milkweed source populations to the center of the species range. According to the “range-center” hypothesis (Alexander et al. 2007, Woods et al. 2012), because plant abundance and herbivore pressure is expected to be higher near the range center than range margins, plant defenses are predicted to be humped shaped (peaking at the range center) and palatability to herbivores should be u-shaped (trough at the range center). Woods et al.’s (2012) study did not support this hypothesis as traits were linearly related to latitude. We also find no support for the range-center hypothesis because none of our models supported a curvilinear relationship between plant traits and latitude (i.e., the quadratic term for latitude was never retained in our model-selection procedures). One important implication from our study, and those of De Frenne et al. (2012), Woods et al. (2012), Zhou et al. (2014), is that because latitudinal clines in plant traits are phenotypically plastic and dependent on climatic conditions, future climate change may fundamentally alter latitudinal gradients in nature. Understanding the biogeography of plant–herbivore interactions in the face of climate change is going to be a daunting task if latitudinal clines in species–interaction traits are universally plastic as these studies suggest.

Phenotypic plasticity in defense and palatability traits

Hypothesis 3: Invasive genotypes are more phenotypically plastic than native genotypes.

Hypothesis 4: Plasticity varies with latitude.

Hypothesis 5: Latitudinal clines are phenotypically plastic.

A fundamental question in the field of invasion biology is what traits promote invasion success. Phenotypic plasticity is thought to be beneficial to founding populations by increasing niche breadth (e.g., Richards et al. 2006, Chun 2011, Davidson et al. 2011) or plasticity may be a trait that evolves in response to the novel environments (Richards et al. 2006, Lavergne and Molofsky 2007). In agreement with the meta-analysis by Davidson et al. (2011), we found that invasive genotypes were 2.5 times more plastic than native genotypes (supporting Hypothesis 3). Because we prevented these genotypes from evolving in response to the biotic and/or abiotic environment (see *Methods*: Plant defense, nutritional,

and palatability traits in common gardens), these differences in trait expression between gardens are attributed to phenotypic plasticity. We also found that trait plasticity varied linearly with latitude (supporting Hypothesis 4), although interestingly, it was primarily for the native genotypes (four of six traits for the native and one of six traits for the invasive genotypes). Similar linear relationships between latitude and plasticity have been reported elsewhere (e.g., Maron et al. 2004, De Frenne et al. 2011, Woods et al. 2012). It is possible that native genotypes under stress from high thermal limits or high herbivore pressure (Cronin et al. 2015) near the southern range limit of the lineage, had to evolve to be more plastic, supporting Chevin and Lande’s (2011) argument that plasticity should be greater at the range margins. In contrast, the southern range limit for invasive *P. australis* is the Gulf of Mexico and geographic barriers may limit their southern range, not high temperature. Coupled with low levels of herbivory, the invasive genotypes may not be as stressed as native genotypes at lower latitudes. Finally, as we have discussed previously (*Trait variation between replicate common gardens*), we found evidence that, for a specific lineage, latitudinal clines differed between gardens, supporting Hypothesis 5 that clines are phenotypically plastic. Latitudinal cline plasticity was evident for total phenolics, water content, percent nitrogen, and C:N ratio (see Fig. 2). Latitudinal cline plasticity appears to be a common occurrence in studies involving multiple common gardens (see Colautti et al. 2009). However, this is the first study to show clinal plasticity at the sub-specific level.

Implications for invasion success

*Hypothesis 6: Across a broad latitudinal range in North America, European genotypes of *P. australis* are successful invaders because they are better defended and less palatable to herbivores than native genotypes.*

Enemy release, i.e., reduced pressure by natural enemies in the introduced relative to the native range (Keane and Crawley 2002), is one of the more prominent hypotheses invoked to explain invasion success (e.g., Mitchell and Power 2003, Liu and Stiling 2006). Biogeographic studies comparing enemy pressure on a plant species in its native and invaded range support this hypothesis (e.g., Mitchell and Power 2003, Liu and Stiling 2006). However, invasion success is also likely to be dependent on the invasive species being less vulnerable to natural enemies than sympatric native species (i.e., “local enemy release”; Zheng et al. 2012), and support for this outcome is mixed (Colautti et al. 2004, Chun et al. 2010). Our field work with the European genotypes of *P. australis* revealed that not only was herbivory significantly lower in their invaded than native range, but also that they suffer 70–650% less herbivory in North America as compared to North American native genotypes (Cronin et al. 2015). In addition, our common-garden study

demonstrates an underlying genetic basis to lower levels of herbivory of European genotypes of *P. australis*: they are less palatable to *H. pruni* and *S. frugiperda*. Invasive genotypes had aphid colonies that were 91% smaller, and had *S. frugiperda* that consumed 54% less leaf material and exhibited 38% lower growth rates than native genotypes (supporting Hypothesis 6). Contrary to Hypothesis 6, we did not find evidence that leaf toughness and total phenolics, our two putative measures of *P. australis* resistance, were greater for invasive than native genotypes. In fact, native genotypes had more total phenolics (Appendix S3: Table S4). One possible explanation for the lower palatability of invasive plants is that leaf water content is 8% lower than for native plants. High water content is beneficial to many insect species (e.g., Huberty and Denno 2004). Finally, not only are invasive genotypes of *P. australis* less palatable to herbivores than native genotypes, they are also more plastic with regard to the traits that likely influence palatability, including total phenolics, water content, percent nitrogen, and C:N ratio. Flexible genotypes may not only be an adaptation to a variable environment (e.g., Agrawal 2001) but may also make it more difficult for herbivores to adapt to its host or track host resources in space (Denno 1983). Given the levels of herbivory observed for *P. australis* in North America, and the potential impact on plant fitness (Lambert and Casagrande 2007, Lambert et al. 2007, Cronin et al. 2015, 2016, Bhattarai et al. 2016), the invasive genotypes of *P. australis* are at a significant advantage over native genotypes. It is no surprise that wetland communities in North America did not resist invasion by European genotypes of *P. australis*.

There is an important biogeographic component to enemy-release for invasive *P. australis*. A strong negative relationship between latitude and herbivory for the native genotypes but no latitudinal gradient for the invasive genotypes results in the strength of enemy release being greater at lower than higher latitudes (Cronin et al. 2015). Although it remains to be tested, we hypothesized that the likelihood of establishment and rate of spread of invasive *P. australis* genotypes should be greatest at lower latitudes (but see Bhattarai and Cronin 2014). We also suggested that non-parallel gradients in herbivory between sympatric native and invasive species or genotypes are likely to be a common occurrence, especially for recent invaders.

Based on our replicated common-garden study, the biogeography of this plant–herbivore interaction is much more complicated than previously envisioned. Latitudinal clines in traits potentially important to the interaction between *P. australis* and its herbivores evolved quickly (≈ 100 yr) for the invasive genotypes, there is plasticity in the regulation of those clines, and in a number of cases, the clines run counter to those for the native genotypes. All of this adds up to substantial heterogeneity in the interactions between native and invasive *P. australis* and their shared herbivores. For example, Bhattarai (2015) found significant variability

along the Atlantic Coast of the United States in the strength of apparent competition (mediated by their shared herbivores) between native and invasive *P. australis* genotypes. Because studies with replicate common gardens often have found similar plasticity in latitudinal clines of invasive species (Richards et al. 2006, Woods et al. 2012, Zhou et al. 2014), we argue that large-scale plant invasions are characterized by considerable spatial heterogeneity in herbivore impact on native and invasive species (or genotypes). This heterogeneity may also explain why support for local enemy release is inconsistent among studies (see e.g., Colautti et al. 2004, Chun et al. 2010).

Concluding remarks

Although this study was conducted at the sub-specific level (i.e., lineages of the same species), we strongly believe that our results and conclusions are applicable to any native-invasive plant system, whether the participants differ at the species, genus or higher taxonomic level. If anything, we would have expected differences in plant traits, clines and plasticity at the sub-specific level to be much more difficult to detect than at higher taxonomic levels. As such, our study suggests that these biogeographic differences between native and invasive taxa, particularly early on in the establishment and spread of invasive taxa, are likely to be common and significant. Lastly, by conducting our study at the sub-specific level, phylogenetic differences between native and invasive taxa that could underlie differences in biogeographic patterns, are less likely to be an issue. This represents one of the great strengths of the *P. australis* study system and is one reason why we consider this a model system for studying species invasions (Meyerson et al. 2016).

Finally, replicate garden studies have proven to be very informative to understanding plant–herbivore interactions. Those positioned in climatically different environments or at different locations within the species range have been especially fruitful (e.g., Woods et al. 2012). Continued research in this area should focus on experimental manipulations at key locations within, at the boundary, and beyond the invasion range, particularly focusing on the impact of natural enemies (herbivores and pathogens) on local plant fitness, the role of generalist and specialist natural enemies, interactions with other sympatric native and invasive plant species and higher trophic levels. As our most pernicious invaders typically have broad invasion fronts, the biogeography of their interactions with native species cannot be ignored if we wish to understand what has led to their success.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecm.1233/full>

DATA AVAILABILITY

Data associated with this paper have been deposited in Dryad: <https://doi.org/10.5061/dryad.r8d1m>