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

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

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

34
35 *Keywords:* *biotic resistance, enemy-release hypothesis, Hyalopterus pruni, invasive species,*
36 *latitudinal gradients, phenotypic plasticity, Phragmites australis, plant-insect interactions, plant*
37 *defense, Spodoptera frugiperda.*

38

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 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 non-native 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 years) 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.

69 The evolution of latitudinal clines in defenses and/or palatability traits in an invasive
70 species may have important implications for invasion success (Bezemer et al. 2014, Cronin et al.
71 2015). For example, the biogeographic paradigm described previously predicts that native plants
72 should exhibit latitudinal clines in defense and palatability traits (see e.g., Pennings et al. 2001).
73 However, an invasive species may not have had sufficient time to evolve a gradient that parallels
74 the gradients for co-occurring native species. These non-parallel gradients in defense or
75 palatability between native and invasive species, particularly early in the invasion process, may
76 create large-scale heterogeneity in the relative impact of herbivores on co-occurring native and
77 invasive plant taxa. As such, in some regions, herbivory may be greater on invasive than native
78 plants (supporting the biotic resistance hypothesis; Levine et al. 2004, Chun et al. 2010) and in
79 other regions, the reverse may occur (i.e., biotic susceptibility). In the only example on this
80 subject, Cronin et al. (2015) found latitudinal gradients in herbivore pressure for native
81 genotypes of *Phragmites australis* (Poaceae) in the field in North America. Invasive genotypes
82 that are sympatric with native genotypes exhibited no latitudinal gradients in herbivore pressure.
83 These non-parallel gradients in herbivory between native and invasive *P. australis* resulted in
84 greater herbivore pressure on native than invasive genotypes in the south (supporting the local
85 enemy-release hypothesis; Zheng et al. 2012) but no difference in herbivore pressure in the
86 north. Importantly, the field study by Cronin et al. (2015) did not allow us to assess whether the
87 geographic variation in *P. australis*-herbivore traits was genetically based, the result of plastic
88 responses by the plants to an environmental gradient (e.g., climate), or some combination of the
89 two.

90 Phenotypic plasticity, variability in the expression of traits in different environments, is
91 another possible mechanism that can generate spatial heterogeneity in native-invasive plant
92 responses to herbivores. Phenotypic plasticity is thought to be beneficial to invaders because
93 plastic “general-purpose genotypes” could have a fitness advantage in founder populations (e.g.,
94 Richards et al. 2006, Chun 2011, Davidson et al. 2011). Alternatively, plasticity may also evolve
95 in an invasive species in response to the novel environment after colonization (Richards et al.
96 2006, Lavergne and Molofsky 2007). In their meta-analysis, Davidson et al. (2011) found strong
97 support for the prediction that invasive species are more phenotypically plastic than native
98 species. Moreover, theoretical models suggest that trait plasticity may vary along a climatic
99 gradient, being greater at range margins than range interiors (Chevin and Lande 2011). A number

100 of studies have demonstrated latitudinal gradients in trait plasticity (e.g., Maron et al. 2004, De
101 Frenne et al. 2011, Woods et al. 2012). Consequently, we may expect co-occurring native and
102 invasive species to differ in trait plasticity along a latitudinal gradient which can contribute to
103 spatial heterogeneity in invasion success. To date, no study has addressed this possibility.

104 Although the common reed, *Phragmites australis*, is native and widespread in North
105 America, a continent wide invasion by introduced European genotypes of *P. australis* in North
106 America has been underway for at least 150 years (Saltonstall 2002). We conducted experiments
107 in replicate common gardens, one in the north (41.49°, University of Rhode Island) and one in
108 the south (30.35°, Louisiana State University), to assess whether genetic-based latitudinal
109 gradients exist for different traits associated with the interactions between native and invasive *P.*
110 *australis* and its herbivores. Because the study was conducted in a common-garden environment
111 and maternal effects were minimized, any latitudinal patterns found would be genetically based.
112 Using multiple native and invasive genotypes of *P. australis* collected across a 17° latitudinal
113 range in North America (Fig. 1), we quantified plant defense levels (leaf toughness, total
114 phenolics) and nutritional condition (percent carbon, percent nitrogen, CN ratio, water content),
115 and conducted experiments to assess palatability to two common and widespread generalist
116 herbivores, the mealy-plum aphid, *Hyalopterus pruni* (Homoptera: Aphididae), and the fall
117 armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). We tested the following six
118 hypotheses. (1) Genetic-based latitudinal clines for plant defenses and palatability to herbivores
119 are evident for native *P. australis* genotypes. (2) Because European invasive genotypes may not
120 have had sufficient time to evolve in response to their novel environment, latitudinal clines in
121 defense and palatability traits will be absent or weak, and thus non-parallel to the gradients for
122 the native genotypes. Based on differences between replicate common gardens, we also tested
123 the hypotheses that (3) invasive genotypes are more phenotypically plastic than native
124 genotypes, (4) plasticity varies with latitude, and (5) latitudinal clines for each lineage differ
125 between gardens, indicating that these clines are phenotypically plastic. Finally, in light of our
126 findings with regard to the previous hypotheses, we test the hypothesis that (6) across a broad
127 latitudinal range in North America, European genotypes of *P. australis* are successful invaders
128 because they are better defended and less palatable to herbivores than native genotypes. Support
129 for this latter hypothesis would suggest that native communities have low biotic resistance to
130 invasion by European *P. australis*.

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METHODS

133 Study system

134 *Phragmites australis* is considered a model organism for the study of plant invasions (for
135 a detailed discussion, see Meyerson et al. 2016). *Phragmites australis* is a tall and robust
136 perennial grass of coastal and freshwater marshes with a nearly worldwide distribution (Marks et
137 al. 1994, Lambertini et al. 2006). It was an uncommon species of wetland communities in North
138 America for millennia but exhibited dramatic and rapid spread over the past ~150 years,
139 particularly in the mid-Atlantic region of North America (Chambers et al. 1999). The rapid
140 spread is attributed to the introduction of an invasive Eurasian genotype in mid 1800s (Chambers
141 et al. 1999, Saltonstall 2002). Additional introduced genotypes have been discovered from the
142 Atlantic and Gulf Coast regions of North America (Lambertini et al. 2012, Meyerson and Cronin
143 2013). At least 14 genotypes of native *P. australis* lineage are distributed throughout North
144 America (Saltonstall 2002, Meadows and Saltonstall 2007) and have been reported to be
145 threatened by the spread of invasive *P. australis* genotypes (Meyerson et al. 2010). Phylogenetic
146 analysis revealed that native and introduced genotypes belong to two different clades (hereafter,
147 lineages) often regarded as separate subspecies (Saltonstall 2002). Hybridization is shown to
148 occur between native and invasive genotypes in nature (Saltonstall 2003, Paul et al. 2010,
149 Saltonstall et al. 2014).

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

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Plant defense, nutritional and palatability traits in common gardens

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We established replicate common gardens at Louisiana State University, Baton Rouge, LA (LSU: 30.35°, -91.14°) and the University of Rhode Island, Kingston, RI (URI: 41.49°, -71.54°, 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 below) 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 (Saltonstall 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 in 19 liter 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 upon detection of leaf yellowing and sprayed regularly with Safer® insecticidal soap (Woodstream Corp., Lititz, PA) to protect them from unwanted herbivores. Safer soap was used because it has a very short (< 2 week) 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 below were conducted with stems

193 that had no visible herbivore damage or traces. We therefore expected little to no effects of
194 background herbivores on plant growth and fitness traits.

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

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

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

221 Because the phenological state of *P. australis* is likely to affect plant physiology,
222 nutritional condition, defense levels, and herbivory (e.g., Liu et al. 2011, Lehndal and Agren
223 2015), our palatability experiments were designed to minimize differences in plant phenology

224 between gardens and among source genotypes within gardens. However, phenology is a difficult
225 concept to apply to grasses in the middle of their growing season because they are constantly
226 producing new shoots. For example, within a pot of *P. australis* during late spring or summer,
227 there is considerable variability in plant phenological state. Consequently, metrics such as
228 growing degree days (GDD; McMaster and Wilhelm 1997) have limited value in these cases.
229 Therefore, to minimize variation among experimental plants, we did the following. First, the
230 experiments were timed to coincide with when the aphid colonies were well established and *S.*
231 *frugiperda* caterpillars were present in the region. Second, we chose mid-size (growing) stems
232 that were between 0.75 and 1.25 m in height. Finally, within a stem, we chose the uppermost,
233 fully open leaf for *H. pruni* or the upper 5-10 leaves for *S. frugiperda* cages.

234 *Palatability to H. pruni.* To assess the palatability of *P. australis* genotypes to aphids, we
235 caged aphids on plants and measured colony growth. This bioassay (and other palatability
236 metrics described below) integrates the effects of multiple plant defensive and nutritional traits
237 on the herbivore's survival and reproduction (i.e., fitness) and is therefore a more holistic
238 measure of plant resistance (Kim 2014). The source of aphids was a naturally occurring stand of
239 *P. australis* within 80 km of each common garden. For ethical reasons and to minimize the
240 genetic variation among aphids within each garden, we used a single source population of aphids
241 for each garden. Cages for the aphid experiment were constructed using five cm lengths of clear
242 acrylic tubing (2.8 cm in diameter, 0.1 cm in thickness) that were inserted over the *P. australis*
243 leaves. The ends of the tubes were sealed with closed-cell foam plugs that had a small fine-
244 screen window cut into their centers for air circulation (see Appendix S2: Fig. S1). Aphid
245 colonies were initiated with two adult aphids caged on the youngest fully open leaf on a
246 randomly selected stem from each pot. Aphids reproduce parthenogenetically and produce a
247 colony within a few days. After 10 d, leaves with aphid colonies were collected, transported on
248 ice to the laboratory, and stored in a freezer at -20° C. With a suitable host, aphid colonies can
249 increase in size by 100-fold in 10 d without any evidence of leaf deterioration or resource
250 depletion due to intraspecific competition (G.P. Bhattarai personal observation). Aphids per
251 colony were enumerated and then dried at 40°C for two days. Dry mass of each colony was
252 determined using a Mettler microbalance (0.1 mg precision). Because aphid mass was strongly
253 correlated with aphid colony size ($R = 0.878$, $P < 0.0001$) we used only colony size in
254 subsequent analyses. Aphid colony survival was determined as the proportion of cages per *P.*

255 *australis* source genotype that had a viable aphid colony after 10 d. Survivorship and colony size
256 were used as indicators of plant palatability to the aphids. The aphid experiment was conducted
257 at LSU from April 13-23, 2012 with nine introduced and five native genotypes (20 cages per
258 genotype), and repeated at URI from June 17-27, 2012 with 12 introduced and six native
259 genotypes (8-12 cages per genotype). The experiments were performed two months later in the
260 URI than LSU garden so that plants were in comparable developmental stages (about two
261 months old) in each garden.

262 *Palatability to S. frugiperda.* For *S. frugiperda*, we also performed a caging experiment
263 and measured the performance of individual larvae feeding on plants from each source genotype.
264 For both gardens, *S. frugiperda* were obtained as eggs from the same source (Benzon Research
265 Inc., Carlisle, PA) and larvae were reared in the laboratory on artificial diet provided by
266 Southland Products (Lake Village, AR). At the fourth instar stage, the mass of each larva was
267 determined, and those larvae within 20-50 mg were selected for the experiment. Sleeve cages
268 (45×60 cm² or 60×75cm² in size) were built using fine insect netting. The cage was inserted over
269 the upper portion of a stem (containing 5-10 leaves) and enclosed around the stem at the bottom
270 using a cable tie (see Appendix S2: Fig. S2). A single caterpillar was released into each cage
271 through a hole cut open in the top of the cage. The hole was subsequently stapled closed. Within
272 each pot, a single stem was selected at random for a cage. The experiment was terminated after 8
273 d, before any caged plants had all available leaf material consumed by the *S. frugiperda* and
274 before the larva could mature to the pupal stage. Each larva was collected, transported on ice to
275 the laboratory and its fresh mass was determined using a Mettler microbalance. Larval growth
276 was calculated as proportional change in fresh biomass during the experiment (\ln final mass [mg]
277 - \ln initial mass [mg]). Survivorship of larvae on each source genotype of *P. australis* was
278 determined as the proportion of cages with a live larva at the end of the experiment. All the cases
279 in which the larva died before the termination of the trial were excluded from the analyses for
280 palatability traits related to *S. frugiperda* (see below).

281 We took photographs of all the remaining leaves inside the cage to quantify leaf area
282 consumed by each larva. Using ImageJ (Rasband 2014), we quantified the remaining leaf area
283 (cm²) for each plant and estimated pre-consumption leaf area by extrapolation. Leaf area
284 consumed by each larva was estimated as the pre-consumption leaf area – leaf area remaining
285 after 8 d. We used leaf area consumed and not proportion leaf area consumed because the total

286 leaf area within a cage was variable and abundant (never being completely consumed), and
287 therefore, the former measure is more informative. The amount of leaf area consumed by larvae
288 could be an indicator of plant defense levels (e.g., Coley 1986). However, herbivores may also
289 consume more to compensate for the lower quality of leaf tissues (Mattson 1980). Finally, we
290 determined biomass conversion efficiency of larva (larval growth per unit [\ln] area of leaf
291 consumed). Leaf area measurements for plants with dead or missing larvae were excluded from
292 the analyses. These four variables, larval survivorship and growth, leaf area consumed, and
293 biomass conversion efficiency, were used as the measurements of plant palatability to chewing
294 herbivores. The experiment was performed on May 23-31, 2013 including nine native and 13
295 invasive genotypes (20 plants per genotype) at LSU garden, and on August 7-15, 2013 including
296 six native and eight invasive genotypes (7-10 plants per genotype) at URI.

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

310 Leaf toughness (force [kg] required to push a blunt steel rod [4.8 mm in diameter]
311 through the leaf) and total phenolics concentration were our measures of plant defenses against
312 herbivores. As a member of the Poaceae, *P. australis* defenses are likely limited to structural
313 defenses and phenolics (McNaughton 1979, Tschardtke and Greiler 1995, Strauss and Agrawal
314 1999). In our field surveys (Cronin et al. 2015), leaf toughness and phenolics were negatively
315 related to leaf-area consumed by chewing herbivores and aphid density, respectively. Using a
316 penetrometer (Itin Scale Co., Inc., Brooklyn, NY), toughness was measured for the fully open

317 uppermost leaf from a randomly selected stem per pot. Leaf toughness was also measured for the
318 top-most leaf inside each cage of the *S. frugiperda* experiment in 2013. Total phenolics (nM/g of
319 dried leaf tissue) were estimated using a modified version of the Folin-Ciocalteu method
320 (Waterman and Mole 1994, Cronin et al. 2015).

321

322

Statistical methods

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

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

345 To help normalize data distributions and homogenize variances among categories (i.e.,
346 gardens, lineages), % carbon, % nitrogen, CN ratio, total phenolics and leaf area were *ln*-
347 transformed. Quantile-quantile plots were also used to identify potential outliers in the

348 distribution of trait estimates. However, in no case did the removal of these data points
349 qualitatively change the conclusions of the model.

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

360 Candidate models were ranked by AICc from lowest to highest value and AICs with a Δ_i
361 value ($= \text{AICc}_i - \text{AICc}_{\min}$) of ≤ 2 were deemed to have substantial support (Burnham and
362 Anderson 2010). We also report the AICc weights (w_i) which indicate the weight of evidence (as
363 a proportion) in favor of model i being the best model given the set of candidate models.
364 Goodness of fit of each mixed effects model was reported as marginal (R^2_m , variance explained
365 by fixed effects) and conditional R^2 (R^2_c , variance explained by the entire model) that are
366 comparable in interpretation to the coefficient of determination, R^2 , for linear models (Nakagawa
367 and Schielzeth 2013). All analyses were run in R 3.2.0 (R Development Core Team 2015) using
368 lme4 (Bates et al. 2014) and AICcmodavg (Mazerolle 2015) packages.

369 The AICc-best models for each trait were used to evaluate the hypotheses outlined in the
370 Introduction. The presence of a latitude effect in the model would suggest that there is a genetic-
371 based latitudinal cline in *P. australis* (Hypothesis 1). The addition of a lineage \times latitude
372 interaction in the best model would further indicate the existence of non-parallel latitudinal
373 gradients between the native and invasive lineage (Hypothesis 2). Otherwise, the absence of this
374 interaction term would suggest parallel latitudinal gradients (or, more appropriately, no evidence
375 that gradients differ between lineages). A garden effect in the best model would suggest
376 phenotypic plasticity in the trait (Hypothesis 3) and a garden \times latitude interaction would support
377 Hypothesis 4 that the degree of plasticity for a trait (i.e., the difference between gardens) varies
378 with latitude of origin of the plants. A three-way interaction between fixed effects (Garden \times

379 Lineage \times Latitude) would support our Hypothesis 5 that the slopes of the latitudinal clines for
380 each lineage differs between gardens (i.e., the latitudinal gradient for each lineage is
381 phenotypically plastic). Finally, if a lineage effect is present in the best model and plant defense
382 trait values are higher or palatability is lower for the native than invasive lineage, it would
383 support the biotic-resistance hypothesis (Hypothesis 6).

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

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

399 For the second objective, we quantified phenotypic plasticity in defense, nutritional and
400 palatability traits for *P. australis* genotypes that were present in both common gardens (4 native,
401 8 invasive; Appendix S1). We could not assess plasticity in palatability to aphids because the
402 aphids were obtained from different source populations located nearby each garden. As such,
403 differences in palatability to aphids between gardens could also be due to genetic differences
404 between aphid source populations (e.g., local co-adaptation between southern aphids and
405 southern populations of *P. australis*). Plasticity for each trait and lineage was measured as the
406 proportional difference in mean trait expression between gardens; e.g., effect size = [mean leaf
407 toughness for native genotypes at LSU – mean leaf toughness for native genotypes at URI]/mean
408 leaf toughness for native genotypes at LSU. Lineage means per garden for each trait were
409 obtained from the least-squares means from the linear mixed-effects model outlined above (or

410 the alternative models for larval survivorship, and leaf toughness) that contained garden and
411 latitude as fixed effects and genotype as a random effect. For all traits, the effect size for native
412 lineage (x -axis) was plotted against the effect size for invasive lineage (y -axis). If the data point
413 for a trait falls above the 1:1 line, it would indicate that plasticity for that trait is greater for the
414 invasive than native lineage. Data points below the line would indicate the opposite. A pairwise
415 t -test was performed to assess whether plasticity for the native and invasive lineages differed for
416 all traits combined (Hypothesis 3). Finally, we also computed plasticity for each source genotype
417 of *P. australis* and used ANCOVA to examine the effects of lineage and latitude on plasticity for
418 each defense and nutritional trait (Hypothesis 4).

419

420

RESULTS

Defense and nutritional traits

422 The phenotypic expression of *P. australis* defense and nutritional traits was strongly
423 influenced by plant lineage and latitude of origin, and also was strongly modulated by where the
424 study was conducted (LSU or URI). The best model, based on AICc weights, included latitude
425 for all six traits (leaf toughness, total phenolics, water content, % nitrogen, % carbon, and CN
426 ratio) and lineage for four traits (total phenolics, water content, % nitrogen, and CN ratio)(Table
427 1, Appendix S3). Interestingly, in all cases where lineage was present in the best model, so was a
428 lineage \times latitude interaction; an indication that native and invasive lineages exhibit nonparallel
429 genetically based gradients in these traits. Finally, not only was the garden where the study was
430 conducted important in all cases (Table 1, see Appendix S3: Table S1 for detailed information),
431 there were many interactions involving garden, lineage and latitude of origin.

432 The defensive trait leaf toughness was negatively related to latitude, decreasing by 51%
433 between our southernmost and northernmost genotypes (Fig. 2A, Table 1). Plants from the LSU
434 garden exhibited steeper declines in toughness with increasing latitude than plants from the URI
435 garden (garden \times latitude interaction). Because of the garden \times latitude interaction in the best
436 model, we conducted separate model-selection procedures for each garden to explore the effects
437 of lineage, latitude and their interactions on leaf toughness. At the LSU garden, native and
438 invasive lineages had parallel latitudinal clines in leaf toughness (no lineage \times latitude
439 interaction in the AICc-best model; Appendix S3: Table S2). There was a significant negative
440 relationship between toughness and latitude for the invasive genotypes but the relationship was

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

448 Similar to total phenolics, the AICc best models to explain water content, % nitrogen and
449 the CN ratio were the same and included all main factors and their 2- and 3-way interactions
450 (Table 1, Appendix S3). AICc weights for these models (the likelihood of the model given the
451 candidate models considered) were > 0.93 . Water content was 8% higher for native than invasive
452 genotypes and 4% higher for plants grown at URI vs. LSU (Fig. 2C, Appendix S3: Tables S4 and
453 S5). At LSU, water content decreased with increasing latitude for both lineages but the
454 latitudinal clines were non-parallel (lineage \times latitude interaction in the AICc-best model;
455 Appendix S3: Tables S2 and S3). At URI, the invasive genotypes exhibited a significant negative
456 latitudinal cline in water content but no cline was evident for the native genotypes. The absence
457 of a lineage \times latitude interaction in the AICc-best models suggest that these two clines are not
458 different (Appendix S3: Table S2). Lineage, latitude and their interaction were important factors
459 contributing to variation in % nitrogen at URI but were unimportant to % nitrogen at LSU (Fig.
460 2D, Appendix S3: Table S2). At the URI garden, % nitrogen increased significantly with latitude
461 for the native genotypes but declined (non-significant) with latitude for the invasive genotypes
462 (non-parallel cline; Appendix S3: Table S3). Overall, % nitrogen was similar between the native
463 and invasive genotypes ($3.13 \pm 1.04\%$ and $3.10 \pm 1.02\%$, respectively; mean \pm SE). For CN ratio,
464 the slopes of the latitudinal clines at URI were in the opposite direction as for % nitrogen and
465 was only significant for the native genotypes (Fig. 2F, Appendix S3: Tables S2 and S3). The
466 difference in CN ratio between lineages changed between gardens (garden \times lineage interaction;
467 Fig. 2F): the CN ratio for invasive genotypes was 12.1% greater at LSU and 13.3% less at URI
468 than for native genotypes. Finally, the AICc best model for % carbon included only garden,
469 latitude and their interaction (Table 1, Appendix S3). Mean % carbon was similar between native
470 and invasive lineages ($45.46 \pm 1.01\%$ and $45.25 \pm 1.00\%$, respectively). On average, the %
471 carbon declined by 1.7% from the south to the north and the relationship was only evident for the

472 plants at the LSU garden (hence the garden \times latitude interaction; Fig. 2E, Appendix S3: Table
473 S2). Only for the invasive lineage at LSU was there a significant latitudinal cline (Appendix S3:
474 Table S3).

475
476 *Palatability to herbivores*

477 Traits associated with *P. australis* palatability to *H. pruni* and *S. frugiperda* were strongly
478 influenced by the garden in which the study was conducted and plant lineage, and to a lesser
479 extent latitude. For aphid colony size, the best model included lineage, latitude, garden and a
480 garden \times lineage interaction (AICc weight = 1.0, Appendix S3: Table S1). Aphid colony size
481 was strongly affected by lineage and latitude at both gardens (both lineages; parallel negative
482 latitudinal gradients) (Fig. 3A, Appendix S3: Tables S2 and S3). The garden \times lineage
483 interaction was present in the model because there was a 26-fold difference in colony size
484 between native and invasive genotypes at LSU but only a 5-fold difference at URI (Fig. 3A).
485 After accounting for the garden effect, aphid colony size averaged 11 times larger for native than
486 invasive genotypes (Appendix S3: Table S4). Based on paired measurements obtained from *P.*
487 *australis* stems, aphid colony size was positively correlated with water content in both gardens
488 (LSU: $R = 0.39$, $P < 0.01$; URI: $R = 0.23$, $P = 0.04$; Appendix S4) and total phenolics in the LSU
489 garden ($R = 0.53$, $P < 0.01$; Appendix S4). No other nutritional or defense trait was correlated
490 with this palatability measure.

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

502 For the four traits associated with *P. australis* palatability to *S. frugiperda*, the main
503 sources of variation in the AICc-best models were garden and lineage (Appendix S3). For all
504 traits, native genotypes were more palatable than invasive genotypes to *S. frugiperda* larvae.
505 Larval growth rate, survivorship, leaf-area consumed and biomass conversion efficiency were
506 61%, 11%, 114% and 33% higher on native than invasive genotypes, respectively (Fig. 3C-F,
507 Appendix S3: Table S4). Interestingly, LSU plants were more palatable than URI plants. After
508 accounting for the effects of lineage, larvae grew 142% larger, had 17% higher survivorship,
509 consumed 65% more leaf tissue, and were 58% more efficient at converting plant biomass to
510 larval biomass on LSU than URI plants (Fig. 3C-F, Appendix S3: Table S5). With regard to
511 biomass conversion efficiency, the difference between lineages was only evident in the URI
512 garden (garden \times lineage interaction) and the relationship between conversion efficiency and
513 latitude was affected by both lineage and garden (lineage \times latitude and garden \times latitude
514 interactions). At the LSU garden, there was no latitudinal gradient in conversion efficiency (Fig.
515 3F, Appendix S3: Table S2). However, at the URI garden, conversion efficiency declined with
516 increasing latitude but only for the invasive genotypes (but no lineage \times latitude interaction; Fig.
517 3F, Appendix S3: Tables S2 and S3). Finally, *S. frugiperda* palatability was generally negatively
518 correlated with leaf toughness and positively correlated with % nitrogen (Appendix S4). The
519 correlations were strongest in the LSU garden where leaf toughness was significantly negatively
520 related to larval growth rate ($R = -0.21$, $P < 0.01$) and leaf-area chewed ($R = -0.20$, $P < 0.01$) and
521 nitrogen was significantly positively correlated with biomass conversion efficiency ($R = 0.40$, P
522 $= 0.04$). At URI, the direction of the relationships between leaf toughness and % nitrogen and *S.*
523 *frugiperda* palatability were similar to those found in the LSU garden but only one relationship
524 was significant - larval growth rate and % nitrogen ($R = 0.60$, $P = 0.05$). Interestingly, water
525 content was negatively correlated with leaf-area chewed in the LSU garden ($R = -0.26$, $P = 0.04$).

526

527 *Plasticity in trait responses*

528 We found strong evidence that plant traits associated with herbivory in *P. australis* were
529 phenotypically plastic, more so for invasive than for native genotypes. Averaged across all 10
530 traits (aphid traits were excluded; see Methods), mean \pm SE plasticity (proportional difference
531 between the two gardens) for the invasive genotypes was 0.23 ± 0.11 and for the native was 0.09
532 ± 0.08 ; a statistically significant 156% difference ($t_9 = 2.708$, $P = 0.024$, Fig. 4). These large

533 differences in trait expression between gardens, for both lineages, likely underlie the ubiquitous
534 garden effect in the previous analyses (Appendix S3: Table S1).

535 Irrespective of *P. australis* lineage, plants reared at the URI garden were more nutritious
536 and less well defended than those at LSU. Plants reared at URI had 7% greater leaf water content
537 and had 24% more nitrogen than those at LSU, whereas plants at LSU produced 131% tougher
538 leaves, 58% more total phenolics and 23% greater CN ratio than plants at URI (Appendix S5).
539 Despite the higher defenses and lower nutrition at LSU, plants grown at LSU were more
540 palatable to herbivores than those at URI. Aphid colony size was 57% greater at LSU than at
541 URI (Appendix S5). *S. frugiperda* larvae consumed two times more leaf area and exhibited a
542 244% higher growth rate at LSU than in URI (Appendix S5). Finally, biomass conversion
543 efficiency of larvae was 137% greater at LSU than URI (Appendix S5).

544 When trait plasticity was measured for each *P. australis* genotype, we found that it varied
545 with latitude for 4 of 6 traits in native genotypes and only 1 of 6 traits in invasive genotypes (Fig.
546 5, Appendix S6). For native genotypes, plasticity in leaf toughness, water content, and %
547 nitrogen decreased (Fig. 5A,C,D) and plasticity in the CN ratio increased (Fig. 5F) with
548 increasing latitude of origin. For the invasive genotypes, leaf toughness was the only trait in
549 which plasticity varied with latitude – a relationship that paralleled the one for native genotypes
550 (Fig. 5A). The difference between lineages in the relationship between plasticity and latitude for
551 total phenolics, water content, % nitrogen and CN ratio are indicated by a significant lineage \times
552 latitude interaction in the model ANCOVAs (Appendix S6). We also examined whether these
553 latitudinal trends were driven by invasive genotypes collected from southern latitudes beyond the
554 distributional range of native genotypes. Re-analyses of these data excluding those genotypes
555 (collected from the sites south of 36° latitude) did not qualitatively alter the results.

556

557

DISCUSSION

558 The juxtaposition of plant-species invasions with large-scale gradients in herbivore
559 pressure and native plant-herbivore interactions is an important yet mostly unexplored issue in
560 the field of invasion biology (Bezemer et al. 2014, Cronin et al. 2015). Our study is the first to
561 demonstrate genetic-based latitudinal clines for traits related to plant-herbivore interactions
562 involving sympatric invasive and native species (or lineages of the same species). These data
563 suggest that clines in the invasive genotypes of *P. australis* evolved within the past ~ 100 years.

564 In 36% of the cases where clines were evident, the clines for native and invasive lineages were
565 not parallel. Moreover, our study showed that the occurrence and direction of latitudinal clines in
566 plant traits was commonly dependent on where the study was conducted (LSU or URI), strongly
567 suggesting that environmental context is an important driver of the expression of plant traits and
568 clines in those traits. Until now, this phenotypic plasticity in latitudinal clines for traits related to
569 species interactions has never been reported. Finally, we found that invasive genotypes were 2.5
570 times more plastic than native genotypes but the native genotypes were much more likely to
571 exhibit significant latitudinal variability in phenotypic plasticity than the invasive genotypes.
572 Overall, this study suggests that traits associated with *P. australis* interactions with its herbivores
573 are under strong genetic and environmental controls and they vary between co-occurring native
574 and invasive *P. australis* genotypes across their latitudinal range in eastern North America. Such
575 spatial heterogeneity within and between lineages with respect to their interactions with
576 herbivores has the potential to generate substantial spatial heterogeneity in biotic
577 resistance/susceptibility that can have important implications for the establishment and spread of
578 invasive genotypes and species.

579

580 *Latitudinal clines in plant-herbivore traits*

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

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

586

587 *Genetic-based clines.* In our study, support for the hypotheses that native genotypes
588 should be more likely to exhibit genetic-based latitudinal clines than invasive genotypes
589 (Hypotheses 1 and 2) was mixed. At least one cline was evident for each defense and nutritional
590 trait, and for at least one trait associated with palatability to *H. pruni* and *S. frugiperda*.
591 However, between the two gardens, there were twice as many latitudinal clines in plant-
592 herbivore traits for the invasive genotypes compared to the native genotypes (8 vs 4 clines,
593 respectively; Appendix S3: Table S3). Moreover, in only one instance was a latitudinal cline for
594 a particular trait evident for both lineages (water content).

595 Although it was strongest in the LSU garden, leaf toughness of native and invasive
596 genotypes generally increased with decreasing latitude. As herbivore pressure in naturally
597 occurring patches of *P. australis* is higher at lower latitudes (Cronin et al. 2015), our common-
598 garden study supports the presumed role of this trait as a defense against herbivores (e.g., Raupp
599 1985, Salgado and Pennings 2005). Its role as a defense is further supported by the finding that
600 palatability to *S. frugiperda* was generally negatively correlated with leaf toughness (see below).
601 Our results with leaf toughness are also consistent with the prediction that longer lifespans of
602 leaves in areas with longer growing seasons should favor tougher leaves (Coley and Aide 1991,
603 Salgado and Pennings 2005). The lone positive latitudinal cline for total phenolics, evident for
604 native genotypes at URI, was the opposite of what we observed in the field (Cronin et al. 2015)
605 and is counter to our expectations if this trait is related to herbivore defense. However, total
606 phenolics represent a broad class of compounds that serve other purposes for plants including
607 protection against photodamage (Close and McArthur 2002). Moles et al. (2011) reported that ca.
608 30% of the published studies found a significant latitudinal gradient in plant total phenolics.
609 Interestingly, the majority of those significant gradients were in the direction observed for the
610 native genotypes from the URI garden. Finally, these results are also consistent with the findings
611 from the meta-analysis of common-garden studies by Colautti et al. (2009) in which there was no
612 consistent directionality in latitudinal clines for plant defense traits, and that evidence for clines
613 in the field do not always match up with clines for the same species in the common garden.

614 Latitudinal gradients in foliar nutrient levels are commonly reported (e.g., Siska et al.
615 2002, Reich and Oleksyn 2004, Lovelock et al. 2007, De Frenne et al. 2013, He and Silliman
616 2015). Between gardens, there was a trend toward decreasing % carbon with increasing latitude,
617 the opposite pattern to that observed in nature (e.g., De Frenne et al. 2013). It is possible that
618 plants adapted to the CO₂-rich environments in the north may have evolved to be less efficient at
619 uptaking or utilizing CO₂ for photosynthesis or storing carbon compounds into their tissues than
620 plants from the relatively CO₂-poor environments in the south (see e.g., Denning et al. 1995).
621 For example, plant stomata are known to stay open longer and wider or the density of stomata
622 increase when the concentration of atmospheric CO₂ decreases in order to maintain an adequate
623 CO₂ gradient between the atmosphere and the leaf (Beerling et al. 1998). It is possible that *P.*
624 *australis* adapted to their local CO₂ concentrations and consequently, southern plants were more
625 efficient at producing or storing carbon compounds. Alternatively, carbon-rich tissues at

626 southern latitudes could serve as a defense against greater herbivore pressure in the south (Orians
627 and Milewsky 2007).

628 We found evidence for latitudinal clines for % nitrogen, CN ratio, and water content but
629 they varied between gardens and lineages. Although nitrogen content in coastal wetland plants
630 tends to increase with increasing latitude (Siska et al. 2002, He and Silliman 2015), we found
631 both positive (URI garden) and negative (LSU garden) latitudinal clines for % nitrogen in native
632 *P. australis* genotypes. Similar results were found for the CN ratio and water content. These
633 differences between lineages and gardens clearly indicate that latitudinal clines in *P. australis*
634 traits are phenotypically plastic (Woods et al. 2012; see section “Phenotypic plasticity in defense
635 and palatability traits”).

636 The genetic-based clines for palatability to *H. pruni* (aphid colony growth and
637 survivorship decreased with increasing latitude; although more strongly for the invasive lineage)
638 do not appear to be caused by latitudinal variation in the *P. australis* nutritional and defensive
639 traits. Although aphid colony growth was positively correlated with total phenolics and water
640 content of leaves, neither of those variables was positively correlated with latitude. Latitudinal
641 gradients in palatability to aphids could be explained by the fact that aphid abundance on *P.*
642 *australis* increases with increasing latitude in North America (Cronin et al. 2015). At northern
643 latitudes, strong selection pressure by these herbivores may have resulted in higher resistance or
644 lower palatability.

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

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

667 *Non-parallel latitudinal gradients between native and invasive genotypes.* One interesting
668 and important finding regarding native and invasive genotypes of *P. australis* was that for 20%
669 of the cases (5 of 24; 12 traits \times 2 gardens), the relationship between latitude and trait level was
670 different for native and invasive genotypes from the same garden (in support of Hypothesis 2).
671 We observed non-parallel clines in water content, total phenolics, % nitrogen, and CN ratio (all
672 but the first occurring in the URI garden). In the case of total phenolics, % nitrogen, and CN
673 ratio, the slope of the relationship with latitude differed in sign between the native and invasive
674 genotypes. These results suggest that the two lineages have evolved in different ways to the same
675 environmental gradient. Although there are many studies that have examined latitudinal clines
676 for species in their native and invasive ranges (Colautti et al. 2009), environmental differences
677 make clinal comparisons questionable. Because the native and invasive ranges of *P. australis*
678 overlap on the same continent, our study provides a much stronger example of clinal evolution in
679 invasive taxa. Although co-occurring native and invasive genotypes across a broad latitudinal
680 range is known only for *P. australis*, a number of other species have co-occurring and distinct
681 invasive genotypes or native-invasive hybrids (Ayres et al. 2004, Lavergne and Molofsky 2007,
682 Ciotir et al. 2013). These are potentially fertile systems for the study of cline formation and
683 evolution. These non-parallel latitudinal clines between co-occurring native and invasive
684 genotypes of *P. australis* can result in spatially varying degrees of local enemy release and biotic
685 resistance (see “Implications for invasion success”).

686 *Origin of latitudinal clines.* We contend that the clines described above for the invasive
687 genotypes of *P. australis* must have arisen *de novo* while in North America. A pre-existing cline,

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

703 Our finding that invasive genotypes were twice as likely as native genotypes to exhibit a
704 latitudinal cline for plant-herbivore traits is somewhat surprising. Such a result would suggest
705 that the invasive genotypes in their new range were more evolutionarily responsive to the same
706 environmental conditions faced by the native genotypes. For two main reasons, this seems
707 unlikely. First, local adaptation and cline formation requires limited gene flow among
708 populations distributed along the latitudinal gradient (Slatkin 1985). Populations of native
709 genotypes are quite rare and isolated in comparison to the extremely widespread and abundant
710 invasive populations (Chambers et al. 1999, Saltonstall 2002). The native genotypes would seem
711 much more likely to exhibit local adaptation and genetic isolation-by-distance than invasive
712 genotypes. This prediction is supported by Kettenring and Mock (2012) who found greater
713 genetic homogeneity among invasive than native populations of *P. australis*. Second, the
714 selection pressures from North American herbivores on the invasive genotypes is considerably
715 weaker than on native genotypes. Based on our latitudinal field surveys of herbivory, invasive
716 genotypes suffered 70% to 650% lower levels of herbivory from all herbivore guilds (chewers,
717 gallers, suckers) than native genotypes and that herbivory levels for invasive genotypes did not
718 vary significantly with latitude (Cronin et al. 2015). Moreover, this study demonstrated that two

719 herbivores, *H. pruni* and *S. frugiperda*, had lower growth and survivorship on the invasive
720 genotypes. If anything, the high levels and strong latitudinal gradients in herbivory of the native
721 genotypes should have favored a high frequency of genetic-based latitudinal clines for this
722 lineage.

723

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

736 One possible reason for the substantial differences in trait expression between gardens is
737 that the gardens reside in very different parts of the range of the two *P. australis* lineages. The
738 URI garden is near the center of the coastal range for both native and invasive lineages, whereas
739 the LSU garden is near the southern extent of the invasive lineage and ~700 km south of our
740 southernmost native population. Plants in the southern garden, particularly for the native lineage,
741 may be at their thermal limits (e.g., Drake et al. 2015) which could result in altered expression of
742 genetic-based clines in these plant nutritional traits. Several studies have examined latitudinal
743 clines in plant traits in replicate common-garden experiments located within, at the boundary or
744 beyond the limits of the species range (e.g., De Frenne et al. 2012, Woods et al. 2012, Zhou et al.
745 2014). For example, in their study of plant growth and defense traits in common milkweed
746 (*Asclepias syriaca*), Woods et al. (2012) found a cline in milkweed phenology in their garden at
747 the southern range limit (North Carolina) but no cline in their garden at the northern range limit
748 (New Brunswick, Canada). Woods et al. (2012) also evaluated whether plant defense trait
749 expression was dependent on the proximity of the milkweed source populations to the center of

750 the species range. According to the ‘range-center’ hypothesis (Alexander et al. 2007, Woods et
751 al. 2012), because plant abundance and herbivore pressure is expected to be higher near the
752 range center than range margins, plant defenses are predicted to be humped shaped (peaking at
753 the range center) and palatability to herbivores should be u-shaped (trough at the range center).
754 Woods et al.’s (2012) study did not support this hypothesis as traits were linearly related to
755 latitude. We also find no support for the range-center hypothesis because none of our models
756 supported a curvilinear relationship between plant traits and latitude (i.e., the quadratic term for
757 latitude was never retained in our model-selection procedures). One important implication from
758 our study, and those of De Frenne et al. (2012), Woods et al. (2012), Zhou et al. (2014), is that
759 because latitudinal clines in plant traits are phenotypically plastic and dependent on climatic
760 conditions, future climate change may fundamentally alter latitudinal gradients in nature.
761 Understanding the biogeography of plant-herbivore interactions in the face of climate change is
762 going to be a daunting task if latitudinal clines in species-interaction traits are universally plastic
763 as these studies suggest.

764

765

Phenotypic plasticity in defense and palatability traits

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

767 *Hypothesis 4: Plasticity varies with latitude.*

768 *Hypothesis 5: Latitudinal clines are phenotypically plastic.*

769

770 A fundamental question in the field of invasion biology is what traits promote invasion
771 success. Phenotypic plasticity is thought to be beneficial to founding populations by increasing
772 niche breadth (e.g., Richards et al. 2006, Chun 2011, Davidson et al. 2011) or plasticity may be a
773 trait that evolves in response to the novel environments (Richards et al. 2006, Lavergne and
774 Molofsky 2007). In agreement with the meta-analysis by Davidson et al. (2011), we found that
775 invasive genotypes were 2.5 times more plastic than native genotypes (supporting Hypothesis 3).
776 Because we prevented these genotypes from evolving in response to the biotic and/or abiotic
777 environment (see Methods “Plant defense, nutritional and palatability traits in common
778 gardens”), these differences in trait expression between gardens are attributed to phenotypic
779 plasticity. We also found that trait plasticity varied linearly with latitude (supporting Hypothesis
780 4), although interestingly, it was primarily for the native genotypes (4 of 6 traits for the native

781 and 1 of 6 traits for the invasive genotypes). Similar linear relationships between latitude and
782 plasticity have been reported elsewhere (e.g., Maron et al. 2004, De Frenne et al. 2011, Woods et
783 al. 2012). It is possible that native genotypes under stress from high thermal limits or high
784 herbivore pressure (Cronin et al. 2015) near the southern range limit of the lineage, had to evolve
785 to be more plastic, supporting Chevin and Lande's (2011) argument that plasticity should be
786 greater at the range margins. In contrast, the southern range limit for invasive *P. australis* is the
787 Gulf of Mexico and geographic barriers may limit their southern range, not high temperature.
788 Coupled with low levels of herbivory, the invasive genotypes may not be as stressed as native
789 genotypes at lower latitudes. Finally, as we have discussed previously ("Trait variation between
790 replicate common gardens"), we found evidence that for a specific lineage, latitudinal clines
791 differed between gardens, supporting Hypothesis 5 that clines are phenotypically plastic.
792 Latitudinal cline plasticity was evident for total phenolics, water content, % nitrogen and CN
793 ratio (see Fig. 2). Latitudinal cline plasticity appears to be a common occurrence in studies
794 involving multiple common gardens (see Colautti et al. 2009). However, this is the first study to
795 show clinal plasticity at the sub-specific level.

796

797

Implications for invasion success

798

Hypothesis 6: Across a broad latitudinal range in North America, European genotypes of *P.*

799

australis are successful invaders because they are better defended and less palatable to

800

herbivores than native genotypes.

801

802

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

812 as compared to North American native genotypes (Cronin et al. 2015). In addition, our common-
813 garden study demonstrates an underlying genetic basis to lower levels of herbivory of European
814 genotypes of *P. australis* – they are less palatable to *H. pruni* and *S. frugiperda*. Invasive
815 genotypes had aphid colonies that were 91% smaller, and had *S. frugiperda* that consumed 54%
816 less leaf material and exhibited 38% lower growth rates than native genotypes (supporting
817 Hypothesis 6). Contrary to hypothesis 6, we did not find evidence that leaf toughness and total
818 phenolics, our two putative measures of *P. australis* resistance, were greater for invasive than
819 native genotypes. In fact, native genotypes had more total phenolics (Appendix S3: Table S4).
820 One possible explanation for the lower palatability of invasive plants is that leaf water content is
821 8% lower than for native plants. High water content is beneficial to many insect species (e.g.,
822 Huberty and Denno 2004). Finally, not only are invasive genotypes of *P. australis* less palatable
823 to herbivores than native genotypes, they are also more plastic with regard to the traits that likely
824 influence palatability, including total phenolics, water content, % nitrogen and CN ratio. Flexible
825 genotypes may not only be an adaptation to a variable environment (e.g., Agrawal 2001) but may
826 also make it more difficult for herbivores to adapt to its host or track host resources in space
827 (Denno 1983). Given the levels of herbivory observed for *P. australis* in North America, and the
828 potential impact on plant fitness (Lambert and Casagrande 2007, Lambert et al. 2007, Cronin et
829 al. 2015, Bhattarai et al. 2016, Cronin et al. 2016), the invasive genotypes of *P. australis* are at a
830 significant advantage over native genotypes. It is no surprise that wetland communities in North
831 America did not resist invasion by European genotypes of *P. australis*.

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

840 Based on our replicated common-garden study, the biogeography of this plant-herbivore
841 interaction is much more complicated than previously envisioned. Latitudinal clines in traits
842 potentially important to the interaction between *P. australis* and its herbivores evolved quickly

843 (≈ 100 years) for the invasive genotypes, there is plasticity in the regulation of those clines, and
844 in a number of cases, the clines run counter to those for the native genotypes. All of this adds up
845 to substantial heterogeneity in the interactions between native and invasive *P. australis* and their
846 shared herbivores. For example, Bhattarai et al. (in review) found significant variability along the
847 Atlantic Coast of the United States in the strength of apparent competition (mediated by their
848 shared herbivores) between native and invasive *P. australis* genotypes. Because studies with
849 replicate common gardens often have found similar plasticity in latitudinal clines of invasive
850 species (Richards et al. 2006, Woods et al. 2012, Zhou et al. 2014), we argue that large-scale
851 plant invasions are characterized by considerable spatial heterogeneity in herbivore impact on
852 native and invasive species (or genotypes). This heterogeneity may also explain why support for
853 local enemy release is inconsistent among studies (see e.g., Colautti et al. 2004, Chun et al.
854 2010).

855

856

Concluding remarks

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

869 Finally, replicate garden studies have proven to be very informative to understanding
870 plant-herbivore interactions. Those positioned in climatically different environments or at
871 different locations within the species range have been especially fruitful (e.g., Woods et al.
872 2012). Continued research in this area should focus on experimental manipulations at key
873 locations within, at the boundary, and beyond the invasion range, particularly focusing on the

874 impact of natural enemies (herbivores and pathogens) on local plant fitness, the role of generalist
875 and specialist natural enemies, interactions with other sympatric native and invasive plant
876 species and higher trophic levels. As our most pernicious invaders typically have broad invasion
877 fronts, the biogeography of their interactions with native species cannot be ignored if we wish to
878 understand what has led to their success.

879

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890

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SUPPORTING INFORMATION

1141 Supporting information may be found online at: <http://onlinelibrary.wiley.com/doi/xx>

DATA AVAILABILITY

1144 Data associated with this paper have been deposited in Dryad:
1145 <http://dx.doi.org/10.5061/dryad.r8d1m>

1146 **Table 1.** Best models (based on AICc model-selection procedure for the effects of garden (G),
 1147 lineage (S), and latitude (L) and all possible two- and three-way interactions on each *P. australis*
 1148 plant defense, nutrition and palatability traits. Detailed information about these models is
 1149 provided in Appendix S3: Table S1.

| Dependent variable | Model |
|--------------------------------------|-------------------------|
| <i>Plant defense traits</i> | |
| Leaf toughness | G L G×L |
| Phenolics | G S L G×S G×L S×L G×S×L |
| <i>Plant palatability traits</i> | |
| Water content | G S L G×S G×L S×L G×S×L |
| % nitrogen | G S L G×S G×L S×L G×S×L |
| % carbon | G L G×L |
| C:N | G S L G×S G×L S×L G×S×L |
| Aphid colony size* | G S L G×S |
| Aphid survivorship** | G S L G×S |
| Larval growth | G S |
| Larval survivorship** | G S |
| Leaf area chewed | G S |
| Larval biomass conversion efficiency | G S L G×S G×L |

1150 *Notes:* Nutrient concentrations (% carbon, % nitrogen, C:N, and phenolics) and leaf area chewed
 1151 (cm²) were *ln* transformed.

1152 *Generalized linear mixed effect model (GLMM) with Poisson family was used in the analysis.

1153 **General linear models were used in these analyses.

1154

1155

FIGURE LEGENDS

1156 **Fig. 1.** Map of the location of *P. australis* source genotypes used in this study and the location of
1157 the common gardens at Louisiana State University (LSU) and University of Rhode Island (URI).

1158 **Fig. 2.** Effects of lineage, latitude and garden on defense and nutritional traits of native and
1159 invasive *P. australis* grown in common gardens at Louisiana State University and the University
1160 of Rhode Island. Symbols in the shaded portion of the graph are the least-squares means (\pm SE)
1161 for different lineages in different gardens. The relationship between a plant trait and latitude is
1162 shown by a line fit by least-squares regression, only for cases in which the AICc-best model
1163 included the effect of latitude (either a Latitude or Lineage \times Latitude or Garden \times Latitude).
1164 Thick lines denote significant trait-latitude relationships ($P \leq 0.05$) and thin lines represent non-
1165 significant relationships ($P > 0.05$; see Appendix S3). Slopes (β_{LSU} and β_{URI}) are reported for the
1166 significant relationships.

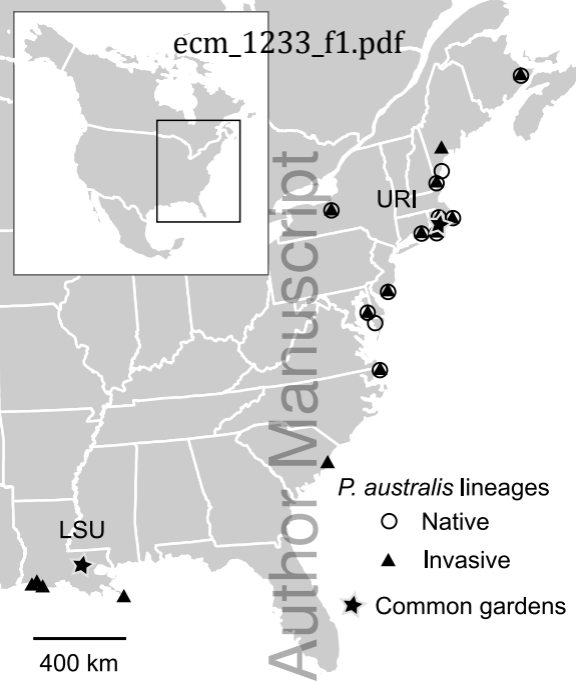
1167 **Fig. 3.** Effects of lineage, latitude and garden on palatability traits of native and invasive *P.*
1168 *australis* grown in common gardens at Louisiana State University and the University of Rhode
1169 Island. Symbols in the shaded portion of each graph are least-squares means (\pm SE) for native
1170 and invasive lineages in each garden. In cases where Latitude or Lineage \times Latitude or Garden \times
1171 Latitude was a factor in the AICc-best model, the relationship between the plant trait and latitude
1172 is shown by a line fit by least-squares regression. Thick lines denote significant trait-latitude
1173 relationships ($P \leq 0.05$) and thin lines represent non-significant relationships ($P > 0.05$; see
1174 Appendix S3). Slopes (β_{LSU} and β_{URI}) are reported for the significant relationships.

1175 **Fig. 4.** Phenotypic plasticity in defense and palatability traits for native and invasive genotypes
1176 of *P. australis*. Plasticity in plant traits ($n = 10$) was estimated as the proportional difference in
1177 mean trait expression between gardens (i.e., [mean trait value for a lineage at LSU – mean trait
1178 value for that lineage at URI]/mean trait value for LSU). Symbols above the 1:1 line depict
1179 greater plasticity in invasive genotypes than the natives. Inset bar graph shows least squares
1180 mean \pm SE plasticity for native (Nat) and invasive (Inv) genotypes. A statistically significant
1181 difference between means (pairwise *t*-test; $P = 0.024$) is represented by an asterisk.

1182 **Fig. 5.** The relationship between trait plasticity (proportional difference in mean trait expression
1183 between gardens) and latitude for native and invasive *P. australis* genotypes. Each point

1184 represents a single genotype that was represented in each garden. Lines are fit by least-squares
1185 regression and were provided only for those traits in which a significant latitude or lineage \times
1186 latitude interaction was detected in an ANCOVA (see Appendix S6). Slopes (β) are reported for
1187 the significant relationships.

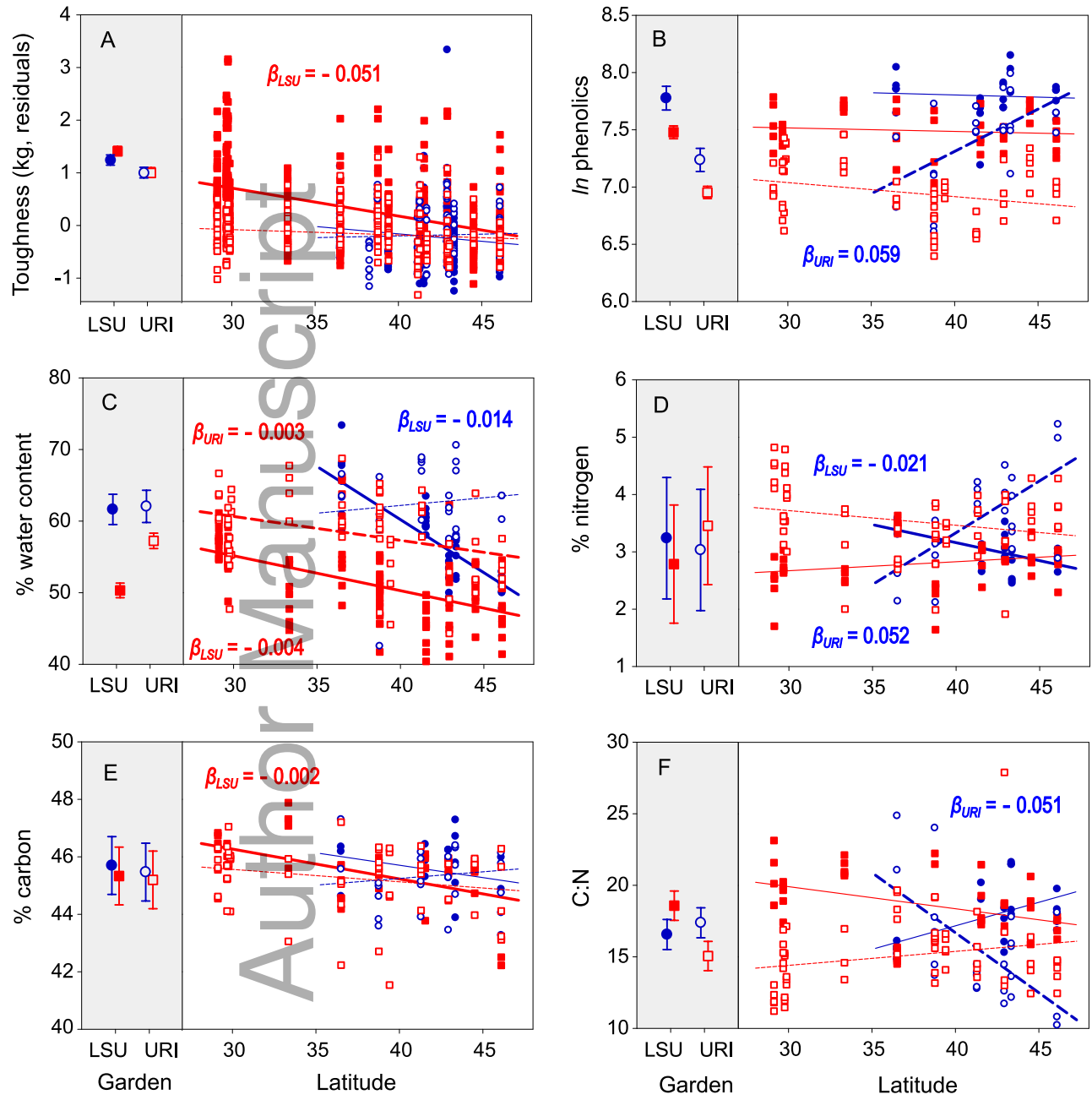
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● Native LSU ○ Native URI ■ Invasive LSU □ Invasive URI

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Figure 2

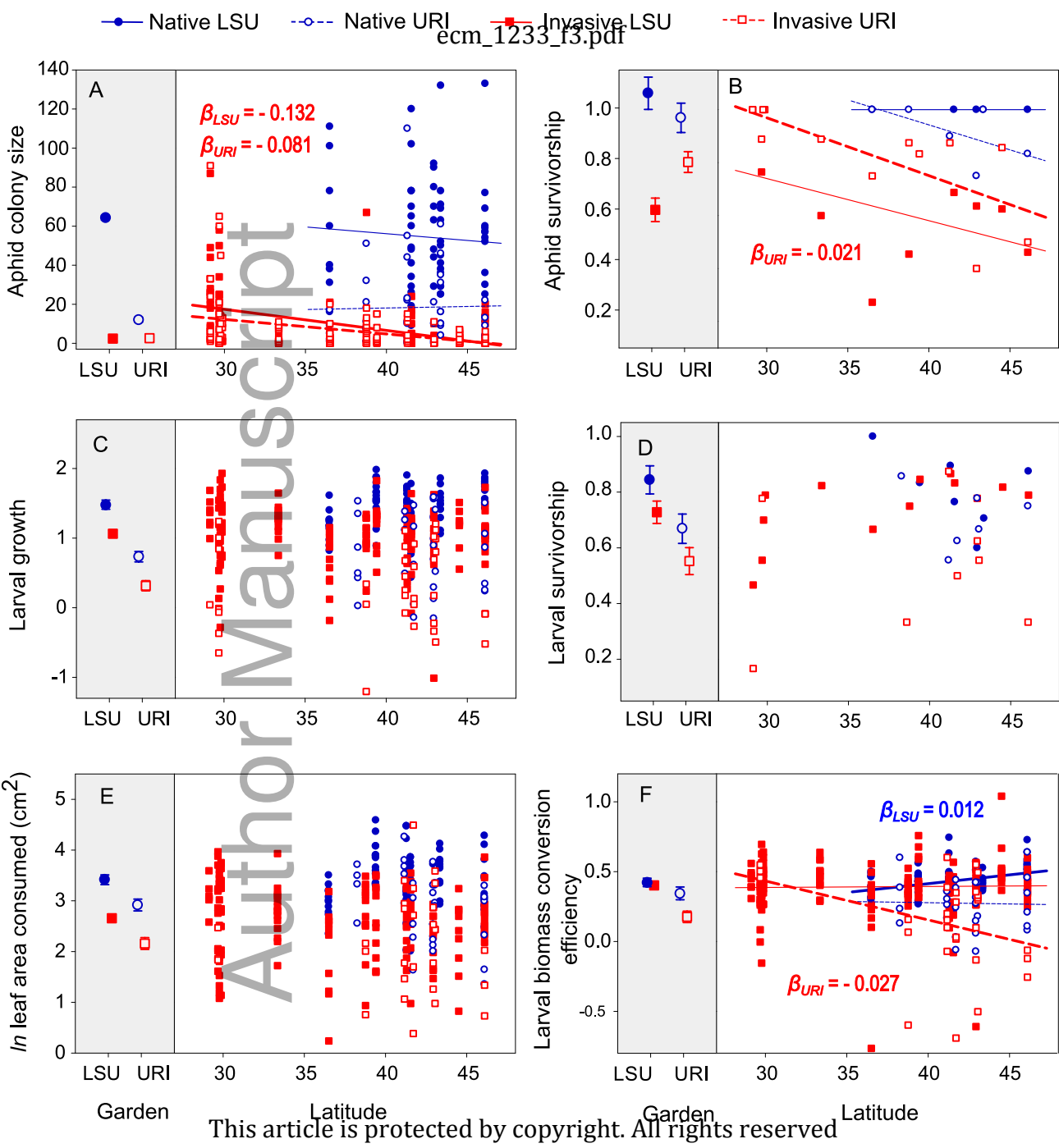
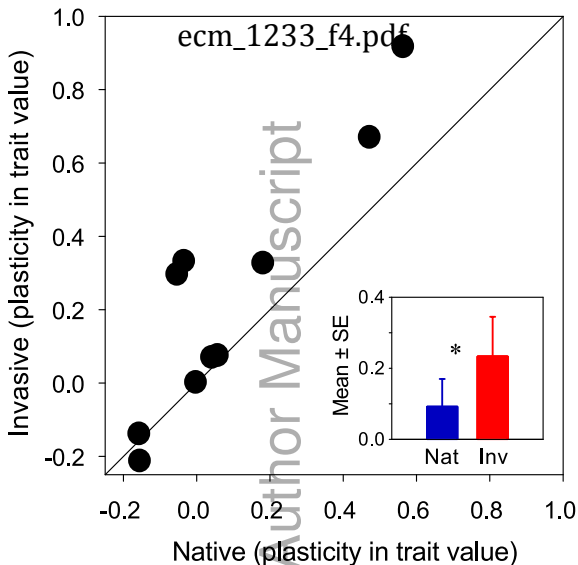
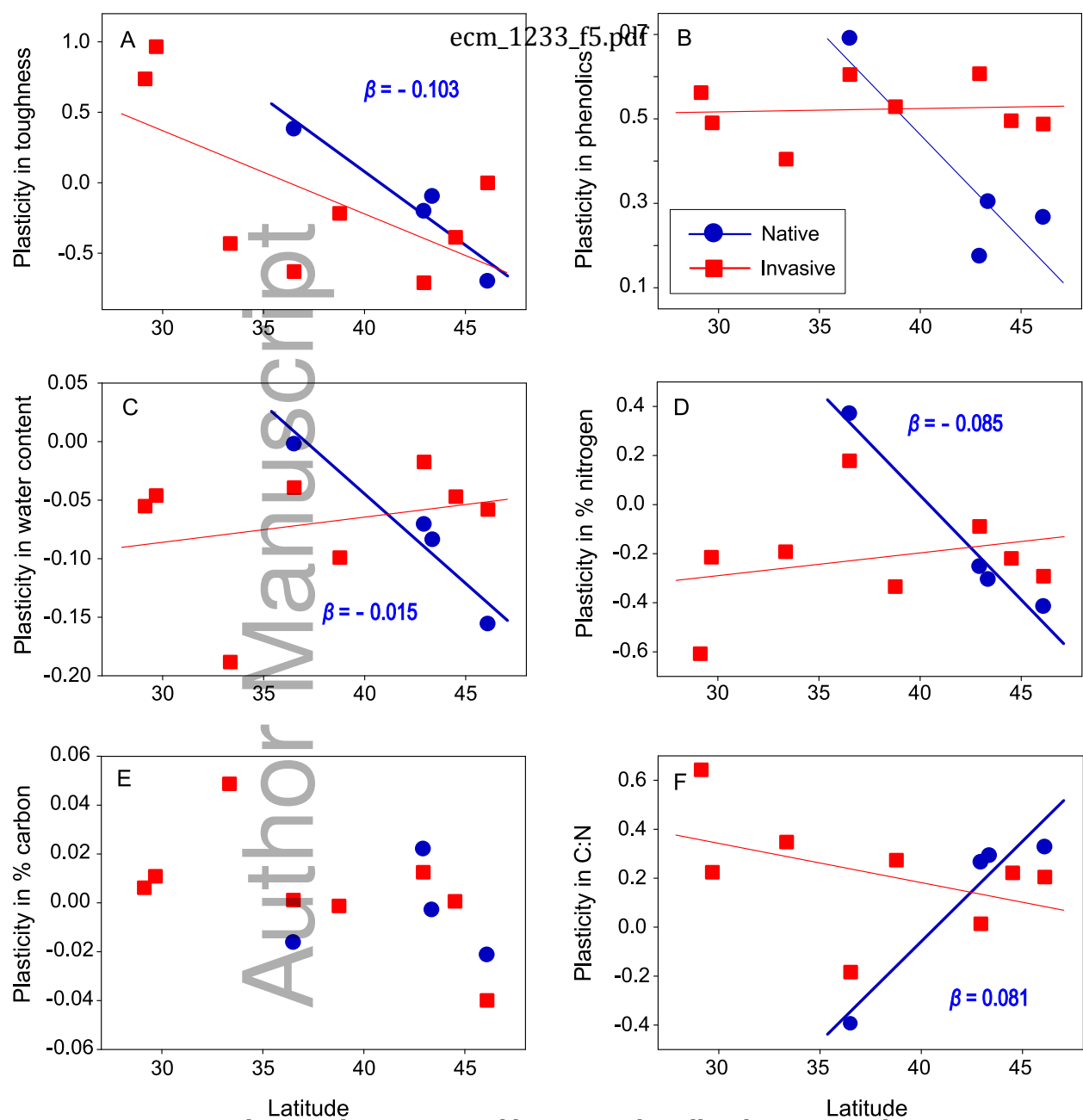


Figure 3



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Figure 4



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Figure 5