Rapid growth and defence evolution following multiple introductions

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Abstract

1. Rapid adaptation can aid invasive populations in their competitive success. Resource-allocation trade-off hypotheses predict higher resource availability or the lack of natural enemies in introduced ranges allow for increased growth and reproduction, thus contributing to invasive success. Evidence for such hypotheses are however equivocal and tests among multiple ranges over productivity gradients are required to provide a better understanding of the general applicability of these theories.
2. Using common gardens, we investigated the adaptive divergence of various constitutive and inducible defence-related traits between the native North American and introduced European and Australian ranges, whilst controlling for divergence due to latitudinal trait clines, individual resource budgets and population differentiation, using >11,000 SNPs.
3. Rapid, repeated clinal adaptation in defence-related traits was apparent despite distinct demographic histories. We also identified divergence among ranges in some defence-related traits, although differences in energy budgets among ranges may explain some, but not all, defence-related trait divergence. We do not identify a general reduction in defence in concert with an increase in growth among the multiple introduced ranges as predicted trade-off hypotheses.
4. *Synthesis*: The rapid spread of invasive species is affected by a multitude of factors, likely including adaptation to climate and escape from natural enemies. Unravelling the mechanisms underlying invasives' success enhances understanding of eco-evolutionary theory and is essential to inform management strategies in the face of ongoing climate change.

Keywords

Latitudinal adaptation, resource allocation, growth-defence trade-offs, EICA, constitutive defence, inducible defence, invasive species, phenolic compounds

Introduction

Biological invasions are occurring at an accelerating pace due to the globalisation of anthropogenic activity (Ricciardi, 2007). Individuals colonizing new ranges likely face environments different from those previously experienced (Sax & Brown, 2000; Allendorf & Lundquist, 2003; Chown *et al.*, 2014). Nonetheless, alien populations often display enhanced performance compared to their native counterparts (Blossey & Notzold, 1995; Thébaud & Simberloff, 2001; Parker *et al.*, 2013), and this can be facilitated by rapid adaptation (Chown *et al.*, 2014; Colautti & Lau, 2015; Dlugosch *et al.*,

2015a). Therefore, in the face of ongoing environmental change, studies on introduced species are imperative to provide insight into invasive success as well as contemporary evolutionary processes.

Resource allocation trade-offs between life-history traits, such as growth rate and reproductive output, feature prominently in evolutionary theories developed to explain the success of invasive species (e.g., Hodgins & Rieseberg, 2011; Kumschick *et al.*, 2013; Turner *et al.*, 2014; Colautti & Lau, 2015). For instance, if abiotic stressors are mitigated upon introduction because of increased resource availability, increased investment in colonization or competitive ability could facilitate invasion success (Grime, 1977; Davis *et al.*, 2000; Bossdorf *et al.*, 2005; He *et al.*, 2010; Dlugosch *et al.*, 2015b). Similarly, the evolution of increased competitive ability hypothesis (EICA) postulates that release from specialist herbivores within the introduced range favours genotypes allocating resources to growth and reproduction in lieu of defence (Blossey & Notzold, 1995). Evidence for such adaptive divergence of invasive populations is however equivocal (Felker-Quinn *et al.*, 2013) perhaps due to allocation trade-offs among multiple competing functions (Mole, 1994; Züst & Agrawal, 2017), variation in resource availability or acquisition (Uesugi *et al.*, 2017; Züst & Agrawal, 2017), interplay with non-adaptive processes (Lee, 2002; Facon *et al.*, 2006; Prentis *et al.*, 2008; Rius & Darling, 2014; Estoup *et al.*, 2016), or other selective factors, such as climate, playing an important role in governing patterns of trait variation within and between ranges (Lachmuth *et al.*, 2011; Turner *et al.*, 2015).

Biotic and abiotic clines impacting plant resistance within ranges (Endara & Coley, 2011; Moles *et al.*, 2011a) can obscure the adaptive underpinnings of trait divergence governed by growth-defence trade-offs in response to changes during invasion in herbivory. For instance, herbivore pressure in the native range is expected to increase towards lower latitudes and potentially drive clines in plant defence in some species (Moles *et al.*, 2011a). This clinal pattern may be absent in the introduced range due to overall lack of herbivory, resulting in non-parallel defence gradients between ranges (e.g. Cronin *et al.*, 2015; Allen *et al.*, 2017). Moreover, high-resource environments support plant species with faster growth that are more vulnerable to herbivores (Coley *et al.*, 1985; Zandt, 2007; Endara & Coley, 2011), resulting in latitudinal clines in defence traits. Latitudinal clines in resource availability could subsequently lead to the evolution of high growth and reduced chemical defences at lower latitudes (Woods *et al.*, 2012; Moreira *et al.*, 2014), although this interspecific pattern may have limited application to intraspecific variation (Hahn & Maron, 2016, but see Woods et al., 2012). However, taken together these patterns suggest that the evolutionary consequences of herbivore escape could change along latitudinal gradients (Blumenthal, 2006). Geographical clines therefore need to be considered in tests of adaptive divergence between ranges (Colautti *et al.*, 2009).

The complex interplay between the evolutionary mechanisms shaping phenotypic divergence could also confound inferences of adaptation. Distinct demographic processes, including founder

effects, genetic drift and admixture, often characterize introduction and alone can lead to divergence between native and introduced populations (Lee, 2002; Facon *et al.*, 2006; Prentis *et al.*, 2008; Rius & Darling, 2014; Estoup *et al.*, 2016). Dissection of the various evolutionary processes that can contribute to trait divergence is required to advance our understanding of rapid spread in invasive species. In addition, the repeatability of evolutionary patterns associated with introductions is unclear, as the majority of studies examining trait evolution following introduction focus on a single invaded range (e.g. Blossey & Notzold, 1995; Joshi & Vrieling, 2005; Hodgins & Rieseberg, 2011; Uesugi & Kessler, 2016, but see Colomer-Ventura *et al.*, 2015). Repeatable trait divergence across multiple invaded ranges would provide support for adaptive divergence of traits during in invasion as well as insight into selective mechanisms contributing to invasion success (Hodgins *et al.*, 2018; van Boheemen *et al.*, 2018).

The frequency and level of attack can impact the evolution of defence traits (Orrock *et al.*, 2015; Bixenmann *et al.*, 2016), which might also be expected to trade off due to their costs and redundancy (Koricheva *et al.*, 2004; Agrawal *et al.*, 2010). Predictable and strong attack should favour constitutive defence, whereas low, infrequent herbivory would favour no, or an inducible response (Agrawal & Karban, 1999; Ito & Sakai, 2009). These responses have been shown to vary over latitudinal clines within ranges (Moreira *et al.*, 2014 Rasmann & Agrawal, 2011). However, the studies exploring evolutionary shifts of constitutive and inducible defences between native and introduced ranges showed mixed results (e.g. Cipollini *et al.*, 2005; Eigenbrode *et al.*, 2008). Various Variable outcomes could result from a decrease in the intensity and frequency of herbivory following introduction (Maron & Vilà, 2001; Agrawal & Kotanen, 2003), including an increase in plasticity (Cipollini *et al.*, 2005; Lande, 2015) or high variability in inducible response among populations (Eigenbrode *et al.*, 2008). Testing such shifts in invasive species would provide insight into factors governing the evolution of induced/constitutive trait defence more generally.

Ambrosia artemisiifolia is a highly suitable system to study adaptive divergence in defence-related traits during invasion. This native North American weed has successfully established globally (Oswalt & Marshall, 2008), including recent introductions to Europe (~160 years ago Chauvel *et al.*, 2006) and Australia (~80 years ago; Palmer & McFadyen, 2012; van Boheemen *et al.*, 2017). Repeated clinal associations were found in *A. artemisiifolia* populations included in the current study, with declines in size and increase in SLA at higher latitudes (van Boheemen *et al.*, 2018), though differences occurred among ranges. At comparable latitudes, European plants were bigger and had lower SLA than natives, while Australian plants had higher SLA leaves (van Boheemen *et al.*, 2018).

We test quantitative trait divergence in 1) physical defence (trichome density), 2) chemical defence (phenolic compounds concentration and richness), and 3) inducibility of chemical defence

among the native North American and introduced European and Australian ranges in a series of common garden experiments. Trichomes are found on the leaves and the stems of plants and deter herbivores (Kessler & Baldwin, 2002; Dalin *et al.*, 2008; Tian *et al.*, 2012). Phenolics are secondary metabolites that are often thought to confer resistance against herbivores (Bhattacharya *et al.*, 2010; War *et al.*, 2012; War *et al.*, 2018). These compounds are also known to be inducible in response to herbivore damage, as well as simulated herbivory treatments including wounding and methyl jasmonate (MeJA) applications (e.g. Lee *et al.*, 1997; Constabel & Ryan, 1998; Keinänen *et al.*, 2001; Heredia & Cisneros-Zevallos, 2009). We accounted for population structure, which could potentially drive patterns in traits that are non-adaptive, using >11,000 double-digest genotype-by-sequencing SNPs. Moreover, we controlled for defence-related trait variation along latitudinal clines.

We predict reduced constitutive defence within the introduced ranges together with elevated inducible response due to lower certainty of attack (Cipollini *et al.*, 2005) and a more plastic (inducible) response in recent colonisations (Lande, 2015). We expect non-parallel defence gradients between native and introduced ranges due to divergence of clines in herbivory (Moles *et al.*, 2011b) and/or variable resource gradients (Blumenthal, 2006; Hahn & Maron, 2016). Finally, we explored the association between defence-related trait divergence and divergence in growth and SLA among ranges as a growth-defence trade-off would result in greater growth in conjunction with reduced defence. However, greater defence could be facilitated by genotypes with enhanced resource acquisition resulting in a positive correlation in traits. By considering the complex interplay of the evolutionary mechanisms impacting defence divergence among multiple ranges, we test evolutionary changes in herbivore defence likely shaped by selection.

Methods

Study species

Ambrosia artemisiifolia is a highly invasive, monoecious, self-incompatible annual plant (Brandes & Nitzsche, 2006), most commonly found in disturbed habitats (Bassett & Crompton, 1975; Lommen *et al.*, 2017) and is expected to expand its range with ongoing climate change (Chapman *et al.*, 2014). It is the leading cause of hayfever worldwide (Taramarcaz *et al.*, 2005) and has a significant impact on crop yields (Kazinczi *et al.*, 2008). Within Europe, admixture following multiple introductions from distinct native sources has been suggested to have contributed to the success of introduced populations, and genetic variation equals levels observed in North America (Chun *et al.*, 2010; Gladieux *et al.*, 2010; Gaudeul *et al.*, 2011; van Boheemen *et al.*, 2017). A subsequent single bottlenecked introduction from Europe has been determined as the origin of the Australian invasion, although the exact European source is unknown (van Boheemen *et al.*, 2017).

Within the native range, around 450 herbivores have been associated with *Ambrosia* species, of which about 30% are specific to the *Ambrosia* genus (Gerber *et al.*, 2011). The North American native specialist *Ophraella communa* is shown to exert high levels of damage (Throop, 2005). Up to 50 polyphagous insect species have been associated with *A. artemisiifolia* in Europe, yet most cause little damage (Gerber *et al.*, 2011; Essl *et al.*, 2015). *Ophraella communa* has been sighted in Southern Switzerland and Northern Italy since 2013 (Müller-Schärer *et al.*, 2014), where it greatly affects *A. artemisiifolia* seedling survival and growth (Cardarelli *et al.*, 2018). In Australia, generalists *Zygogramma bicolorata* (leaf-feeding) and *Epiblema strenuana* (stem-boring) are widespread and seemingly exert some control (Palmer & McFadyen, 2012).

Experimental set-up

To explore the divergence of constitutive quantitative defence traits between native and introduced ranges ("constitutive-defence experiment"), while accounting for divergence along latitudinal clines, we collected *Ambrosia artemisiifolia* seeds in 2013-2014 from broad geographical scales within the native North America and introduced Europe and Australia. We raised seedlings in a common garden (for detailed methods, see Supporting Information). Briefly, we stratified seeds for 6 weeks at 4°C (Willemsen, 1975). After a 2-week germination at 30°C with 12h light/dark cycle, we randomly transplanted the seedlings into 100ml kwikpot trays with Debco mix, followed by a second transplant to 0.7L pots containing Debco and 1.5ml slow-release fertilizer (Osmocote Pro, eight to nine months) one month later. We top-watered all plants and artificially manipulated daylight following the light cycle at the median latitude for all populations (47.3°N). To explore constitutive defence, we selected a seedling from four maternal lines, originating from 28 North American, 32 European and 20 Australian locations (Supporting Information, Table S1).

A separate greenhouse experiment was conducted to test whether the inducibility of defence response varied among plant origins (hereafter, "induction experiment"). We used a subset of populations used in the constitutive experiment (10 North American, 17 European and 12 Australian locations, Table S1). For each population, we selected four maternal lines, and grew two seedlings per line as above. One seedling per maternal line was allocated to either the control or simulated herbivory treatment. We simulated herbivory by vertically cutting off half of the newest fully formed leaf (wounding) and subsequently spraying the whole plant with 1mM methyl jasmonate (MeJA) (Campos-Vargas & Saltveit, 2002; Heredia & Cisneros-Zevallos, 2009; Hodgins & Rieseberg, 2011; Jordan *et al.*, 2015). Control plants were not wounded and were sprayed with distilled water.

Trait measurements

179 For the constitutive experiment, we recorded trichome density at the mid-point of each plant under a
180 dissecting microscope (Olympus, SZ-PT) using a 1 cm x 0.3 cm stem area at the mid-point of each
181 plant, nine weeks after the second transplant. Three weeks later, we scanned one young, fully expanded
182 leaf from each plant and calculated leaf area using ImageJ and the R package LeafArea (Katabuchi,
183 2015). We dried leaves at 45 °C for seven days and an addition 12 hours prior to weighing and weighed
184 to the closest milligram. We calculated specific leaf area (SLA) by dividing leaf area by dry leaf weight
185 (mm^2/mg). We deconstructed plants for biomass measurements once the majority of seeds had ripened.
186 We placed aboveground components in paper bags and dried these in ovens at 45 °C for at least 36
187 hours. Before dry weight biomass measures, we dried materials for an additional minimum of 24 hours
188 to ensure the dry weight was constant at the time of measuring and it was not variable due to humidity
189 in the air or incomplete drying. We weighed this shoot biomass to the closest 0.1 gram.
190 Leaf samples for phenolic analyses were collected four weeks after the second transplant by
191 clipping approximately 200 mg of the newest fully expanded leaf, which was flash frozen in liquid
192 nitrogen and stored in a -80 ˚C. In the induction experiment, we collected leaf samples 24 hours after
193 the final treatment. Samples were extracted in 1 ml of 80% methanol (% by volume in water) using a
194 Qiagen TissueLyser II for 30 seconds at 30 rps twice and centrifuged for 30 minutes at 2700 rpm.
195 Phenolic samples from the constitutive-defence experiment were analysed using HPLC Agilent 1200
196 series (Agilent Technologies Australia, Mulgrave, VIC, Australia) equipped with C18 reverse-phase
197 column (Waters, 5.0 µm, 250 mm x 4.6 mm; Alltech Australia, Baulkham Hills NSW, Australia). The
198 elution system consisting of solvents (A) 0.25% H3PO4 in water (pH 2.2) and (B) acetonitrile was: 0–
199 6 min, 0–12% of B; 6–10 min, 12-18% of B, and 10-30 min, 18-58% of B, with a flow rate of 1 mL/min
200 and injection volume of 15 µL (Keinänen *et al.*, 2001). Samples from the induction experiment were
201 analysed with Agilent Infinity 1260 equipped with C18 reverse-phase column (Poroshell 120 EC-C18,
202 2.7 µm, 150 mm x 3.0 mm; Agilent Technologies Australia, Mulgrave, VIC, Australia). The elution
203 method was modified from above and was: 0–2 min, 0–12% of B; 2–3.3 min, 12–18% of B, and 3.3–
204 10 min, 18-58% of B, with a flow rate of 0.5 mL/min and injection volume of 5 µL. In both
205 experiments, phenolic compound peaks were identified to their compound classes using UV spectra
206 and relative abundance was quantified at 320 nm. To estimate phenolic compound richness, we
207 counted the number of detectable peaks. The relative concentration of eight major phenolic peaks was
208 estimated as area under each peak divided by sample fresh weight. Results could not be directly
209 compared as the two experiments were performed in different greenhouses and samples from each
210 experiment were run using different HPLC machines.
211
212 *Statistical analyses*

To test if constitutive defence differed among ranges (the constitutive experiment), we examined individual phenolic compound composition in a multivariate analysis of covariance (MANCOVA) and the concentration of individual phenolic compounds, phenolic compound richness, total phenolic concentration, and trichome density in univariate mixed models. To account for latitudinal variation within and among ranges, each multi- and univariate model included range, latitude, their interaction and a latitude^2 effect as fixed factors. To control for neutral population structure, possibly shaping trait variation between populations, univariate models included q-values as a random effect, as obtained from STRUCTURE analysis performed on genetic data. For the multi- and univariate analyses, we improved normality of the data by square-root- or log-transforming traits where appropriate. For the MANCOVA, we included the concentration of eight major phenolic compounds (Spearman's ρ among peaks < 0.75) and calculated Wilks' λ (multivariate F-value) to measure the strength of the associations. To measure the variance explained by the fixed effects or the full model within the univariate models, we calculated the marginal and conditional coefficients of determination using the MuMIn package (Bartón, 2018). We computed type III Wald F-values with Kenward-Roger degrees of freedom and step-wise removed non-significant effects, starting with the highest order interaction. For univariate models, we plotted the partial residuals of each response variable by ranges, thus accounting for latitudinal clines and neutral population genetic structure and reported these adjusted means and standard errors for each range, calculated using the *phia* packaged (De Rosario-Martinez, 2015).

To explore the variation in inducibility among ranges (the induction experiment), we repeated the steps for the constitutive experiment, now including treatment and its interactions with range and latitude as fixed effects. For the MANCOVA, we included five peaks (excluding three with Spearman's $\rho > 0.75$) to increase power of this test (Scheiner, 2001). We retained treatment in these models, as this was the variable of interest. Here, a significant treatment effect would signify an inducible response, whereas a treatment x range interaction would imply this response differs between ranges. A treatment x latitude interaction would indicate different inducibility at different latitudes. To test if variation in induction differed between ranges (Eigenbrode *et al.*, 2008), we compared the coefficient of variation (c_v) using the modified signed-likelihood ratio test for equality with 10^4 simulations in the cvequality package (Krishnamoorthy & Lee, 2014; Marwick & Krishnamoorth, 2018).

To examine associations between defence-related traits and plant growth and to assess if divergence in individual resource budgets could have resulted in range differences in defence-related trait investment, we tested responses of phenolic richness, phenolic concentrations or trichome density

246 to shoot biomass or SLA. Each model included a defence-related trait as response, with shoot biomass
247 or SLA, range and their interaction as predictors. We used individual trait values and included
248 individual STRUCTURE q-values and sampling location as random factors. We explored significant
249 range x defence interactions using a Holm p-value correction in the *phia* package (De Rosario-
250 Martinez, 2015). In these models, a negative association between defence-related traits and shoot
251 biomass would suggest a trade-off, while a positive one might indicate differences in resource
252 acquisition. Range differences at similar values of shoot biomass or SLA would indicate defence-
253 related trait divergence independent of genotypic differences in individual resource budgets.
254 To explore if constitutive and inducible defence trade off, we first calculated the induced level
255 of total phenolics for each maternal line as the difference between damage and control treatments of
256 the two half-sibs. This estimate of induction is thought to reduce correlations with control treatment
257 estimates and thus the collinear associations (e.g. due to genotypic biases) will not mask the trade-off
258 associations (Morris *et al.*, 2006). We included population of origin and individual q-values as random
259 factor in these models. A significant negative association between induced and constitutive levels of
260 phenolic concentration and richness would indicate the presence of a trade-off. All statistical analyses
261 were conducted in R v3.4.3 (R Core Team, 2018).
262
263 **Results**
264 *Constitutive defence trait divergence between ranges*
265 We found significant range divergence in constitutive phenolic composition (Table 1a), resulting from
266 differences between the introduced Europe and the native North America ($F_{8,66}$=3.280, p=0.010,
267 Wilks' λ=0.716) (Table 1b). Total phenolic concentration was similar between the native and
268 European populations, but 28% lower in Australia (Table 1b&c, Fig. 1). Phenolic peak richness
269 differed among ranges (Table 1): it was highest in the introduced European range (adjusted mean of
270 43 peaks) followed by the native North American (40 peaks) and introduced Australian ranges (33
271 peaks). Trichome density showed no differences between ranges (Table 1a, Fig. 1). The composition
272 of individual phenolic compounds and peak richness depended on latitude, though no such effect was
273 found for the total phenolic concentration or trichome density (Table 1a, Fig. 2). We did not observe
274 range x latitude interactions for any of the defence-related traits (Table 1a), suggesting latitudinal
275 clines, when present, did not differ between ranges.
276
277 *Inducible defence trait divergence between ranges*
278 We found a significant treatment effect on individual phenolic compound composition in the induction
279 experiment ($F_{5,59}$=12.014, p<0.001, Wilks' λ=0.496) (Table 2). The total phenolic concentration was

280 slightly suppressed in the herbivory-simulating treatment (Table 2, Fig. 3), but the phenolic peak
281 richness did not show a response to experimental treatment (Table 2, Fig. 3). We identified no
282 treatment x range x latitude interactions (Table 2, Fig. 3), suggesting there is no range difference in
283 inducibility clines. Also, the absence of treatment x latitude interactions (Table 2, Fig. 3), suggests an
284 overall lack of latitudinal clines in inducibility. Moreover, no treatment x range interactions (Table 2,
285 Fig. 3) suggests the inducible response did not differ between ranges. We did not find range differences
286 in the variation of inducible phenolic peak richness (c_v=1.401, p=0.496) or concentration (c_v=2.297,
287 p=0.317).
288
289 *Associations between defence, biomass and specific leaf area (SLA)*
290 Within each range, phenolic concentration and richness was positively correlated with shoot biomass,
291 whereas we found a negative association between trichome density and shoot biomass (Table 3, Fig.
292 4). We found high-SLA leaves had lower phenolic concentration and peak richness, yet higher
293 trichome density (Table 3, Fig. 4). No interactions were significant between range and predictor
294 variables (shoot biomass or SLA), suggesting these associations among traits were consistent between
295 ranges (Table 3, Fig. 4). These results emphasize the close relationship between plant growth,
296 physiology and defence.
297 When controlling for shoot biomass or SLA, total phenolic concentration in European plants
298 was higher compared to North American individuals of comparable weight (Table 3, Fig. 4), whereas
299 no difference existed in latitude models (Table 1, Fig. 1). Conversely, phenolic peak richness was no
300 longer significantly different between North America and Europe (Table 3, Fig. 4) compared to range
301 comparisons accounting for latitude (Table 1, Fig. 1). Australian plants exhibited lower phenolic
302 concentration and peak richness compared to native or European plants of comparable weight or SLA.
303 Yet, at the same plant weight, Australian plants had higher trichome densities than in the other ranges
304 (Table 3, Fig. 4). These patterns match previous analyses including latitude (Table 1, Fig. 1).
305
306 *Constitutive-inducible trade-offs*
307 Induced levels of phenolic concentration and richness, the response variables, were negatively
308 associated with the predictors, the constitutive levels (concentration: $F_{1,141.88}$=76.286, p<0.001;
309 richness: $F_{1,123.81}$=78.126, p<0.001; Fig. 5). We found no range differences in either induced response
310 trait (concentration: $F_{2,31.07}$=0.265, p=0.769; richness: $F_{2,31.518}$=1.719, p=0.196; Fig. 5), nor did we
311 identify interactions between range and the predictor constitutive phenolic concentration
312 ($F_{2,141.11}$=0.866, p=0.423). Range x constitutive phenolic peak richness ($F_{2,129.82}$=3.045, p=0.051) was

313 marginally significant. These results suggest that constitutive and inducible defence trade off, although
314 there is no difference between ranges.
315
316 **Discussion**
317 In this study, we found evidence for divergence in defence related traits within and between ranges.
318 Repeated latitudinal clines in phenolic richness and individual phenolic composition were identified,
319 suggesting rapid adaptation of phenolics to local environments following invasion. Though we
320 observed reduced phenolic concentration and richness in introduced Australia compared to the native
321 plants while controlling for genetic structure, levels were similar or slightly higher in the introduced
322 Europe compared to native populations at comparable latitudes and energy budgets. In addition,
323 trichome density did not differ among ranges. These patterns are inconsistent with the Evolution of
324 Increased Competitive Ability (EICA) hypothesis. In line with predictions, however, a trade-off
325 between the constitutive and inducible phenolics was observed together with similar phenolic
326 inducibility among ranges. To our knowledge, this is the first study testing the evolution of defence
327 related traits across multiple introductions while exploring the predicted confounding of latitudinal
328 clines, population substructure or genotypic differences in individual resource budgets. Therefore, the
329 apparent absence of the predicted repeated selection against high defence investment following
330 introduction is unlikely to be entirely masked by these factors. We examine these processes in detail
331 and suggest alternative mechanisms driving defence-trait divergence within and among native and
332 introduced ranges.
333
334 *Divergence in constitutive defence-related traits*
335 The rapid and repeated latitudinal divergence in phenolic compound composition and richness
336 populations suggests direct or indirect selection of latitude-associated factors. Corresponding to our
337 findings, typical reported patterns include high growth and low defence at more productive high-
338 resource (Coley *et al.*, 1985; Zandt, 2007; Endara & Coley, 2011), low-latitude environments (Woods
339 *et al.*, 2012; Moreira *et al.*, 2014; Hahn & Maron, 2016, Blumenthal, 2006). Native clines in herbivore
340 load could result in such observations, though the predicted herbivore reduction following introduction
341 should lead to non-parallel defence clines among native and introduced ranges (Cronin *et al.*, 2015;
342 Allen *et al.*, 2017). However, in our data, latitudinal clines in defence-related traits (phenolic
343 compound composition and peak richness) were parallel, which could reflect consistent patterns of
344 selection with latitude in all three ranges. The absence of the predicted patterns could result from
345 parallel clines in herbivore loads in each range or the presence of alternative evolutionary forces
346 driving latitudinal trait divergence in the multiple ranges. Indeed, clinal variation in herbivory is not

347 as common as previously thought (Moles *et al.*, 2011a)), although geographic information on *A.*
348 *artemisiifolia* herbivore pressure is needed.

349 Alternatively, latitudinal clines could arise through direct selection on the alternative functions
350 of phenolic compounds, or indirect selection through genetic covariance with traits under climate
351 mediated selection. Climate was previously shown to be a more important driver of trait divergence
352 compared to enemy release (Colautti *et al.*, 2009; Colautti & Barrett, 2013; Colomer-Ventura *et al.*,
353 2015). Along these lines, climatic differences between the ranges not captured by latitude could
354 contribute to patterns of divergence in Australian defence-related traits. For instance, trichomes protect
355 plants from UV (Bassman, 2004; Hauser, 2014) and selection for this alternate function in high-UV
356 Australia (WHO, 1998) could potentially explain the higher density of trichomes in this range when
357 controlling for plant size. Herbivore exclusion experiments at various latitudes and environments
358 would be important for to disentangling how resource availability, herbivory and other climatic factors
359 might interact during invasion and impact the evolution of growth and defence traits.

360 When correcting for these latitudinal clines, we found conflicting patterns of defence-related
361 trait divergence between the native and two introduced ranges. Genotypic differences in resource
362 acquisition (Van Noordwijk & de Jong, 1986; Agrawal, 2011; Züst & Agrawal, 2017) and historical
363 contingency (Lee, 2002; Facon *et al.*, 2006; Prentis *et al.*, 2008; Rius & Darling, 2014; Estoup *et al.*,
364 2016) can obscure trade-offs predicted under resource-allocation trade-off hypotheses. Accordingly,
365 we show trichome density, phenolic concentration and peak richness were strongly associated with
366 plant biomass and specific leaf area (SLA)(Fig. 4). Contrary to EICA predictions, phenolic peak
367 concentration was significantly higher in Europe compared to native North America at comparable
368 shoot biomass, although this difference disappeared when controlling for latitude or SLA. Similarly,
369 phenolic richness was significantly higher in Europe than North America at equivalent latitudes, but
370 this likely reflects the larger size and lower SLA of European plants at similar latitudes (van Boheemen
371 *et al.*, 2018). However, lower phenolic concentration and peak richness in Australia was still present
372 at similar latitude, biomass or SLA compared to North America. Invasion history is unlikely a major
373 factor in this observed defence-related trait divergence as we accounted for population genetic
374 structure in our analysis.

375 An adaptive reduction of constitutive defence traits following introduction to Europe and
376 Australia was predicted due to a general release from natural enemies. However, levels of chemical
377 defence-related traits (phenolic concentration and richness) were not consistently lower in introduced
378 ranges compared to native populations. Such unexpected findings could have resulted from variation
379 in contemporary herbivory among introduced ranges. Of particular relevance to the EICA hypothesis
380 are specialist herbivores, as herbivory by specialists, but not necessarily generalists, is hypothesized

381 to consistently decline during invasion (Müller-Schärer *et al.*, 2004; Joshi & Vrieling, 2005; Felker-
382 Quinn *et al.*, 2013). Indeed, introduced Japanese *A. artemisiifolia* populations re-exposed to specialist
383 leaf beetle *Ophraella communa* for >10 years were more resistant than herbivore-free populations
384 (Fukano & Yahara, 2012). However, rapid adaptation to *O. communa* is unlikely to have led to the
385 observed elevated European phenolic concentration and richness, as the seeds used in our experiment
386 were collected in 2014 and this beetle is constrained to southern Europe since introduction in 2013
387 (Sun *et al.*, 2017).

388 Alternatively, differences in generalist load between introduced ranges could have resulted in
389 variation in quantitative digestibility-reducing chemicals (e.g., phenolics), which defend against both
390 generalist and specialists (Müller-Schärer *et al.*, 2004). Surveys describe a high diversity of generalist
391 species in Europe (Gerber *et al.*, 2011; Essl *et al.*, 2015) but not in Australia (Palmer & McFadyen,
392 2012) suggesting herbivory in this species is higher in Europe than Australia. However, Genton *et al.*
393 (2005) previously found that compared to native Ontario, the most common forms of damage (chewing
394 and perforation) together with the generalist herbivore load was reduced in introduced France
395 populations consistent with enemy escape in Europe compared to native North America. Contradicting
396 EICA expectations, but consistent with our findings for Europe, the French plants showed no
397 evolutionary loss of defence (Genton *et al.*, 2005). Therefore, although reductions in both specialists
398 and generalist herbivores have been documented in both introduced ranges, we did not find parallel
399 changes in defence-related traits as predicted by EICA, suggesting such predictions are perhaps too
400 simplistic. Nevertheless, a more detailed survey of herbivory, resistance and the mechanisms of
401 resistance across all three ranges is warranted, particularly given the contrasting patterns of divergence
402 in phenolics identified among the two introduced ranges. Moreover, a more detailed analysis of the
403 alternative functions of these phenolics (e.g., allelopathic interactions and plant structure; Bhattacharya
404 *et al.*, 2010; Li *et al.*, 2010) is required.

405 A key assumption of EICA is a resource allocation trade-off between defence and growth.
406 However, even when these traits have evolved in the EICA predicted direction, negative genetic
407 correlations have yet to be detected (Franks *et al.*, 2008; Schrieber *et al.*, 2017; Hodgins *et al.*, 2018).
408 Furthermore, a direct trade-off might not be evident as resource reallocation from other traits, drawing
409 from the same resource pool, could allow for the elevated investment in defence related traits and
410 growth simultaneously (Züst & Agrawal, 2017; Hodgins *et al.*, 2018). For instance, an analysis of
411 climate niche shifts in *A. artemisiifolia* has revealed that Eurasian and Australasian ranges on average
412 experience warmer, wetter climates compared to the North American range (van Boheemen *et al.*,
413 2018). Therefore, reduced investment in abiotic stress tolerance could have allowed for resource

414 reallocation to defence and growth simultaneously. These recently acknowledged complex dynamics
415 underlying competitive ability call for more integrative tests of invasive spread.
416
417 *Constitutive versus induced range divergence*
418 We observed a negative association between constitutive and inducible defence-related traits
419 suggesting a trade-off (Koricheva *et al.*, 2004; Agrawal *et al.*, 2010). A decrease in the level and
420 predictability of attack in the introduced range is expected to cause a reduction in constitutive defence
421 and the maintenance or increase in inducible defence (Cipollini *et al.*, 2005; Orians & Ward, 2010;
422 Lande, 2015). In agreement with this prediction constitutive phenolic levels were reduced in Australia,
423 while inducible response did not differ among ranges. Such maintenance of mean inducibility could
424 result from insufficient herbivore pressure, where a selection-drift imbalance could increase inducible
425 variability (Eigenbrode *et al.*, 2008). Although analysis of neutral markers suggests genetic drift has
426 been particularly strong in Australia (van Boheemen *et al.*, 2017), we did not reveal any increase in
427 inducible variation. The growing body of literature testing constitutive versus inducible defence in
428 native and introduced ranges frequently report inconsistent results varying from reductions, to
429 maintenance, to increases in either defence (Cipollini *et al.*, 2005; Eigenbrode *et al.*, 2008; Beaton *et*
430 *al.*, 2011; Carrillo *et al.*, 2012; Cipollini & Lieurance, 2012; Wang *et al.*, 2012; Wang *et al.*, 2013;
431 Fortuna *et al.*, 2014; Gu *et al.*, 2014; Agrawal *et al.*, 2015; Macel *et al.*, 2017) and calls for more
432 detailed research on the costs-benefit trade-offs of the various responses.
433 Remarkably, we found evidence of a suppression of phenolics in response to herbivore
434 simulation for some populations, especially those with high constitutive levels, in contrast to some
435 previous studies (Lee *et al.*, 1997; Constabel & Ryan, 1998; Keinänen *et al.*, 2001; Heredia &
436 Cisneros-Zevallos, 2009). Conversely, cardenolide suppression was found in various *Asclepias* species
437 at high constitutive levels (Rasmann *et al.*, 2009), though the mechanistic cause was not discussed
438 (Agrawal *et al.*, 2010). We propose that the retraction of phenolics from damaged leaves could indicate
439 a cost-reducing response when the inducible phenolic compounds have alternative functions
440 (Bhattacharya *et al.*, 2010; Li *et al.*, 2010), or function only in particular aspects of defence response,
441 not induced by the treatment. Similarly, perhaps for those individuals already heavily defended with
442 phenolic compounds increased investment in this chemical defence, which failed to deter an attacking
443 herbivore would have diminishing returns, leading to the potential activation of other defence
444 strategies by the plant. Nevertheless, gaining insight into such cost-benefit associations might prove
445 difficult due to, for instance, issues identifying and addressing all factors influencing the investment
446 of defence-related traits (Neilson *et al.*, 2013).
447

Conclusion

We demonstrate divergence of growth and defence traits within multiple introduced ranges that is consistent with rapid adaptation during introduction. Furthermore, we do not find evidence to support the hypothesis that escape from specialist enemies drives the evolution of increased competitive ability in this invasive, as enhanced growth in European populations was not in lieu of defence-related trait reduction. The evolution of growth and defense traits in Australian populations, derived from European founders, occurred rapidly (~80 generations), seemingly unconstrained by strong genetic bottleneck identified in this range (van Boheemen *et al.*, 2017), as measured traits in these two invaded ranges are primarily on opposing ends of the phenotypic spectrum of values. Evidence is growing that adaptation to climate might explain the alarming spread and success of non-natives to a greater extent than release from natural enemies (Colautti *et al.*, 2009; Colautti & Barrett, 2013; Colomer-Ventura *et al.*, 2015). Indeed, we identified repeated latitudinal patterns in phenolics in all three ranges consistent with climate mediated selection, perhaps through corresponding shifts in the biotic community or through direct or indirect selection on phenolics by climate variables. This study emphasizes that intraspecific multi-introduction tests of trait divergence of invasive species provide important insight into contemporary evolutionary process during range expansion.

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Author contributions

All authors developed the project, with data collection and analyses carried out by LAB and SB, refined by AU and KH. All authors discussed the results, contributed to the MS writing and gave final approval for publication.

Data accessibility

Sequence data are available at the National Center for Biotechnology Information (NCBI) Sequence Read Archive under Bioproject PRJNA449949.

Scripts are available on https://github.com/lotteanna/defence_adaptation.

Data is available on https://doi.org/10.6084/m9.figshare.8028875.v1

Fig. 1. Partial residual defence trait responses (phenolic concentration, peak richness and trichome density) of *A. artemisiifolia* populations to range, accounting for latitudinal clines and neutral population structure. Different letters indicate significance for pairwise range comparisons (Table 1).

Fig. 2. Population mean response of phenolic peak richness to range (native North America, blue triangles; introduced Europe, green squares; introduced Australia, red circles) and latitude in *Ambrosia artemisiifolia,* with predicted latitudinal clines (+/- 95% confidence interval) corrected for neutral population structure.

Fig. 3. Partial residual defence trait responses (phenolic concentration and peak richness) of *A. artemisiifolia* populations to control (solid symbols) and herbivore simulating treatment (wounding + MeJA, dashed transparent symbols), with covariates of range, accounting for latitudinal clines and neutral population structure. Letters indicate significance of effect (Table 2).

Fig 4. Defence trait responses (phenolic concentration, peak richness and trichome density) of *A. artemisiifolia* individuals to range (native North America (blue triangles); Europe (green squares); Australia (red circles)), shoot biomass (left panels) or specific leaf area (right panels) with model predictions (+/- 95% confidence interval, Table 3).

Fig. 5. Inducible (D: wounding + MeJA; C: control) versus constitutive (control) defence trait responses (phenolic concentration and peak richness) of *A. artemisiifolia* populations among ranges (native North America: blue triangles; Europe: green squares; Australia: red circles) with model predictions (+/- 95% confidence interval).

Table 1. *Ambrosia artemisiifolia* defence-related trait responses (population means) to range, latitude, their interaction and latitude^2 (to account for non-linear relationship) in the constitutive experiment in multivariate (individual phenolic compounds) and univariate analyses (a), with dissection of significant range effects ($p<0.05$) in post-hoc tests (b). We reported Wald type III F (a) or χ^2 test values (b), Kenward-Roger degrees of freedom (subscript), significance (symbols). In the multivariate analysis, Wilk's λ measure the strength of the association, in univariate analyses, marginal (R^2m) and conditional (R^2c) coefficients measure the variance explained by fixed effects or full models. Models were step-wise reduced starting with the highest order non-significant interaction and univariate analyses included neutral population genetic structure as a random effect.

*ns: p>0.1; #: p<0.1, *: p<0.05; **: p<0.01; ***: p< 0.001*

a.	Range	Latitude	Latitude^2	Range: Latitude	R^2m	R^2c
Individual phenolic compounds composition	$4.520_{16,132}$***, λ =0.417	$6.928_{8,66}$***, λ=0.544	$2.814_{8,66}$*, λ =0.746	$0.849_{16,128}$(ns), λ =0.817		
Phenolic concentration	$8.601_{1,71.918}$***	$0.934_{1,73.244}$(ns)	$0.036_{1,72.973}$(ns)	$0.127_{1,70.054}$(ns)	0.189	0.290
Phenolic richness	$7.615_{2,58.48}$**	$7.79_{1,71.66}$**	$0.046_{1,53.51}$(ns)	$2.027_{2,69.64}$(ns)	0.177	0.688
Trichome density	$0.663_{2,71.183}$(ns)	$1.825_{1,66.941}$(ns)	$3.148_{1,74.991}$#	$0.121_{2,69.141}$(ns)	0.055	0.055

b.	North America - Europe	North America - Australia	Europe - Australia
Individual phenolic compounds composition	$3.280_{8,66}$**, λ =0.716	$1.580_{8,66}$(ns), λ=0.840	$1.994_{8,66}$(ns), λ =0.805
Phenolic concentration	1.397_{1}(ns)	9.311_{1}**	17.321_{1}***
Phenolic richness	4.783_{1}*	12.725_{1}***	15.843_{1}***

Table 2. *Ambrosia artemisiifolia* defence-related trait responses (population means) to range, latitude, treatment, their interactions and latitude^2 in the inducible experiment in multivariate (individual phenolic compounds) and univariate analyses. Range, latitude, their interaction or latitude^2 were included as covariates and significant results were not explored further. We reported Wald type III F, Kenward-Roger degrees of freedom (subscript), significance (symbols) (a). In the multivariate analysis, Wilk's λ measure the strength of the association, in univariate analyses, marginal (R^2m) and conditional (R^2c) coefficients measure the variance explained by fixed effects or full models (a). Models were step-wise reduced starting with the highest order non-significant interaction and univariate analyses included neutral population genetic structure as a random effect. *ns: p>0.1; *: p<0.05; **: p<0.01; ***: p< 0.001*

	Range	Latitude	Latitude^2	Range:Latitude	Treatment	Treatment:Range	Treatment:Latitude	Treatment: Range: Latitude	R^2m	R^2c
Individual phenolic compounds concentration	$7.591_{10,118}$***, λ=0.370	$10.637_{5,59}$***, λ=0.526	$4.818_{5,59}$**, λ=0.710	$2.311_{10,118}$*, λ=0.699	$12.014_{5,59}$***, λ=0.496	$0.326_{10,112}$(ns), λ=0.944	$0.977_{5,58}$(ns), λ=0.922	$1.357_{10,108}$(ns), λ=0.789		
Phenolic concentration	$4.970_{2,30.905}$*	$5.932_{1,31.505}$*	$1.577_{2,28.556}$(ns)	$1.745_{1,31.505}$(ns)	$4.241_{1,35.628}$*	$0.005_{2,32.285}$(ns)	$1.077_{1,35.428}$(ns)	$1.417_{2,31.192}$(ns)	0.201	0.457
Phenolic richness	$3.764_{2,29.93}$*	$4.700_{1,31.08}$*	$1.340_{2,28.24}$(ns)	$6.030_{1,30.95}$*	$0.850_{1,35.33}$(ns)	$0.091_{2,32.1}$(ns)	$0.825_{1,34.89}$(ns)	$1.923_{2,30.78}$ (ns)	0.323	0.723

Table 3. Constitutive defence trait response of *Ambrosia artemisiifolia* individuals to shoot biomass, specific leaf area and their interaction with range (a), with dissection of significant range effects ($p<0.05$) in post-hoc tests (b). We reported corresponding figure, Wald type III F (a) or χ^2 test values (b), Kenward-Roger degrees of freedom (subscript) and significance (symbols). Marginal (R^2m) and conditional (R^2c) coefficients measure the variance explained by fixed effects or full models (a). Models were step-wise reduced starting with the highest order non-significant interaction and included population origin and neutral population genetic structure as random effects.

*ns: p>0.1; #: p<0.1, *: p<0.05; **: p<0.01; ***: p< 0.001*

a.

Predictor	Response	Figure 4	Range	Predictor	Range:Predictor	R^2m	R^2c
Shoot biomass	Phenolic concentration	A	$19.321_{2,79.435}$***	$31.098_{1,181.299}$***	$1.441_{2,184.26}$(ns)	0.160	0.189
	Phenolic richness	B	$18.96_{2,79.29}$***	$53.389_{1,180.51}$***	$1.565_{2,187.82}$(ns)	0.199	0.224
	Trichome density	C	$10.242_{2,79.4}$***	$18.49_{1,174.06}$***	$0.525_{2,180.84}$(ns)	0.093	0.103
Specific leaf area	Phenolic concentration	D	$6.162_{2,71.167}$**	$38.464_{1,208.912}$***	$0.98_{2,202.53}$(ns)	0.202	0.269
	Phenolic richness	E	$5.349_{2,69.16}$**	$42.692_{1,217.97}$***	$0.32_{2,202.6}$(ns)	0.215	0.331
	Trichome density	F	$1.828_{2,71.7}$(ns)	$10.994_{1,204.66}$**	$1.196_{2,206.12}$(ns)	0.049	0.121

b.

Predictor	Response	Figure 4	North America - Europe	North America - Australia	Europe - Australia
Shoot biomass	Phenolic concentration	A	5.846_1*	22.155_1***	40.087_1***
	Phenolic richness	B	2.546_1(ns)	26.667_1***	37.964_1***
	Trichome density	C	2.255_1(ns)	13.049_1***	21.047_1***
Specific leaf area	Phenolic concentration	D	2.152_1(ns)	5.032_1*	12.485_1**
	Phenolic richness	E	0.459_1(ns)	6.655_1*	10.643_1**
	Trichome density	F	-	-	-

533 **Literature cited**

534 Agrawal, AA (2011). Current trends in the evolutionary ecology of plant defence. *Functional Ecology*,
535 **25**, 420-432.

536 Agrawal, AA, Conner, JK and Rasmann, S (2010). Tradeoffs and negative correlations in evolutionary
537 ecology. Evolution since darwin: The first 150 years. M. A. Bell, D. J. Futuyma, W. F. Eanes
538 and J. S. Levinton. Sunderland, Massachusetts, USA, Sinauer Associates**:** 243-268.

539 Agrawal, AA, Hastings, AP, Bradburd, GS, Woods, EC, Züst, T, Harvey, JA and Bukovinszky, T
540 (2015). Evolution of plant growth and defense in a continental introduction. *The American*
541 *Naturalist*, **186**, E1-E15.

542 Agrawal, AA and Karban, R (1999). Why induced defenses may be favored over constitutive strategies
543 in plants. The ecology and evolution of inducible defenses. R. Tollrian and C. D. Harvell.
544 Princeton, New Jersey, Princeton University Press**:** 45-61.

545 Agrawal, AA and Kotanen, PM (2003). Herbivores and the success of exotic plants: A
546 phylogenetically controlled experiment. *Ecology Letters*, **6**, 712-715.

547 Allen, WJ, Meyerson, LA, Cummings, D, Anderson, J, Bhattarai, GP and Cronin, JT (2017).
548 Biogeography of a plant invasion: Drivers of latitudinal variation in enemy release. *Global*
549 *Ecology and Biogeography*, **26**, 435-446.

550 Allendorf, FW and Lundquist, LL (2003). Introduction: Population biology, evolution, and control of
551 invasive species. *Conservation Biology*, **17**, 24-30.

552 Bartón, K (2018). Multi-model inference. CRAN, The R Foundation for Statistical Computing**:** R
553 package.

554 Bassett, IJ and Crompton, CW (1975). The biology of canadian weeds. *Canadian Journal of Plant*
555 *Science*, **55**, 463-476.

556 Bassman, JH (2004). Ecosystem consequences of enhanced solar ultraviolet radiation: Secondary plant
557 metabolites as mediators of multiple trophic interactions in terrestrial plant communities.
558 *Photochemistry and Photobiology*, **79**, 382-398.

559 Beaton, LL, Van Zandt, PA, Esselman, EJ and Knight, TM (2011). Comparison of the herbivore
560 defense and competitive ability of ancestral and modern genotypes of an invasive plant,
561 *lespedeza cuneata*. *Oikos*, **120**, 1413-1419.

562 Bhattacharya, A, Sood, P and Citovsky, V (2010). The roles of plant phenolics in defence and
563 communication during *agrobacterium* and *rhizobium* infection. *Molecular Plant Pathology*,
564 **11**, 705-719.

565 Bixenmann, RJ, Coley, PD, Weinhold, A and Kursar, TA (2016). High herbivore pressure favors
566 constitutive over induced defense. *Ecology and Evolution*, **6**, 6037-6049.

567 Blossey, B and Notzold, R (1995). Evolution of increased competitive ability in invasive
568 nonindigenous plants: A hypothesis. *Journal of Ecology*, **83**, 887-889.
569 Blumenthal, DM (2006). Interactions between resource availability and enemy release in plant
570 invasion. *Ecology Letters*, **9**, 887-895.
571 Bossdorf, O, Auge, H, Lafuma, L, Rogers, WE, Siemann, E and Prati, D (2005). Phenotypic and
572 genetic differentiation between native and introduced plant populations. *Oecologia*, **144**, 1-11.
573 Brandes, D and Nitzsche, J (2006). Biology, introduction, dispersal and distribution of common
574 ragweed (*ambrosia artemisiifolia* l.) with special regard to germany. *Nachrichtenblatt des*
575 *Deutschen Planzenschutzdienstes*, **58**, 286-291.
576 Campos-Vargas, R and Saltveit, ME (2002). Involvement of putative chemical wound signals in the
577 induction of phenolic metabolism in wounded lettuce. *Physiologia Plantarum*, **114**, 73-84.
578 Cardarelli, E, Musacchio, A, Montagnani, C, Bogliani, G, Citterio, S and Gentili, R (2018). *Ambrosia*
579 *artemisiifolia* control in agricultural areas: Effect of grassland seeding and herbivory by the
580 exotic leaf beetle *ophraella communa*. *NeoBiota*, **38**, 1.
581 Carrillo, J, Wang, Y, Ding, J, Klootwyk, K and Siemann, E (2012). Decreased indirect defense in the
582 invasive tree, *triadica sebifera*. *Plant Ecology*, **213**, 945-954.
583 Chapman, DS, Haynes, T, Beal, S, Essl, F and Bullock, JM (2014). Phenology predicts the native and
584 invasive range limits of common ragweed. *Global Change Biology*, **20**, 192-202.
585 Chauvel, B, Dessaint, F, Cardinal-Legrand, C and Bretagnolle, F (2006). The historical spread of
586 *ambrosia artemisiifolia* l. In france from herbarium records. *Journal of Biogeography*, **33**, 665-
587 673.
588 Chown, SL, Hodgins, KA, Griffin, PC, Oakeshott, JG, Byrne, M and Hoffmann, AA (2014).
589 Biological invasions, climate change and genomics. *Evolutionary Applications*, **8**, 23-46.
590 Chun, YJ, Fumanal, B, Laitung, B and Bretagnolle, F (2010). Gene flow and population admixture as
591 the primary post-invasion processes in common ragweed (*ambrosia artemisiifolia*) populations
592 in france. *New Phytologist*, **185**, 1100-1107.
593 Cipollini, D and Lieurance, DM (2012). Expression and costs of induced defense traits in *alliaria*
594 *petiolata*, a widespread invasive plant. *Basic and Applied Ecology*, **13**, 432-440.
595 Cipollini, D, Mbagwu, J, Barto, K, Hillstrom, C and Enright, S (2005). Expression of constitutive and
596 inducible chemical defenses in native and invasive populations of *alliaria petiolata*. *Journal*
597 *of Chemical Ecology*, **31**, 1255-1267.
598 Colautti, RI and Barrett, SC (2013). Rapid adaptation to climate facilitates range expansion of an
599 invasive plant. *Science*, **342**, 364-366.

600 Colautti, RI and Lau, JA (2015). Contemporary evolution during invasion: Evidence for
601 differentiation, natural selection, and local adaptation. *Molecular Ecology*, **24**, 1999-2017.
602 Colautti, RI, Maron, JL and Barrett, SCH (2009). Common garden comparisons of native and
603 introduced plant populations: Latitudinal clines can obscure evolutionary inferences.
604 *Evolutionary Applications*, **2**, 187-199.
605 Coley, PD, Bryant, JP and Chapin, FS (1985). Resource availability and plant antiherbivore defense.
606 *Science*, **230**, 895-899.
607 Colomer-Ventura, F, Martínez-Vilalta, J, Zuccarini, P, Escolà, A, Armengot, L and Castells, E (2015).
608 Contemporary evolution of an invasive plant is associated with climate but not with herbivory.
609 *Functional Ecology*, **29**, 1475-1485.
610 Constabel, CP and Ryan, CA (1998). A survey of wound-and methyl jasmonate-induced leaf
611 polyphenol oxidase in crop plants. *Phytochemistry*, **47**, 507-511.
612 Cronin, JT, Bhattarai, GP, Allen, WJ and Meyerson, LA (2015). Biogeography of a plant invasion:
613 Plant–herbivore interactions. *Ecology*, **96**, 1115-1127.
614 Dalin, P, Ågren, J, Björkman, C, Huttunen, P and Kärkkäinen, K (2008). Leaf trichome formation and
615 plant resistance to herbivory. Induced plant resistance to herbivory. A. Schaller, Springer**:** 89-
616 105.
617 Davis, MA, Grime, JP and Thompson, K (2000). Fluctuating resources in plant communities: A
618 general theory of invasibility. *Journal of Ecology*, **88**, 528-534.
619 De Rosario-Martinez, H (2015). Phia: Post-hoc interaction analysis (v0.2-1). CRAN, The R
620 Foundation for Statistical Computing**:** R package.
621 Dlugosch, KM, Anderson, SR, Braasch, J, Cang, FA and Gillette, HD (2015a). The devil is in the
622 details: Genetic variation in introduced populations and its contributions to invasion. *Molecular*
623 *Ecology*, **24**, 2095-2111.
624 Dlugosch, KM, Cang, FA, Barker, BS, Andonian, K, Swope, SM and Rieseberg, LH (2015b).
625 Evolution of invasiveness through increased resource use in a vacant niche. *Nature Plants*, **1**,
626 1-5.
627 Eigenbrode, SD, Andreas, JE, Cripps, MG, Ding, H, Biggam, RC and Schwarzländer, M (2008).
628 Induced chemical defenses in invasive plants: A case study with *cynoglossum officinale* l.
629 *Biological Invasions*, **10**, 1373-1379.
630 Endara, MJ and Coley, PD (2011). The resource availability hypothesis revisited: A meta-analysis.
631 *Functional Ecology*, **25**, 389-398.

632 Essl, F, Biró, K, Brandes, D, Broennimann, O, Bullock, JM, Chapman, DS, Chauvel, B, Dullinger, S,
633 Fumanal, B and Guisan, A (2015). Biological flora of the british isles: *Ambrosia artemisiifolia*.
634 *Journal of Ecology*, **103**, 1069-1098.
635 Estoup, A, Ravigné, V, Hufbauer, R, Vitalis, R, Gautier, M and Facon, B (2016). Is there a genetic
636 paradox of biological invasion? *Annual Review of Ecology, Evolution, and Systematics*, **47**, 51-
637 72.
638 Facon, B, Genton, BJ, Shykoff, J, Jarne, P, Estoup, A and David, P (2006). A general eco-evolutionary
639 framework for understanding bioinvasions. *Trends in Ecology & Evolution*, **21**, 130-135.
640 Felker-Quinn, E, Schweitzer, JA and Bailey, JK (2013). Meta-analysis reveals evolution in invasive
641 plant species but little support for evolution of increased competitive ability (eica). *Ecology*
642 *and Evolution*, **3**, 739-751.
643 Fortuna, TM, Eckert, S, Harvey, JA, Vet, LE, Müller, C and Gols, R (2014). Variation in plant defences
644 among populations of a range-expanding plant: Consequences for trophic interactions. *New*
645 *Phytologist*, **204**, 989-999.
646 Franks, SJ, Pratt, PD, Dray, FA and Simms, EL (2008). Selection on herbivory resistance and growth
647 rate in an invasive plant. *The American Naturalist*, **171**, 678-691.
648 Fukano, Y and Yahara, T (2012). Changes in defense of an alien plant *ambrosia artemisiifolia* before
649 and after the invasion of a native specialist enemy *ophraella communa*. *PloS One*, **7**, e49114.
650 Gaudeul, M, Giraud, T, Kiss, L and Shykoff, JA (2011). Nuclear and chloroplast microsatellites show
651 multiple introductions in the worldwide invasion history of common ragweed, *ambrosia*
652 *artemisiifolia*. *PLoS One*, **6**, e17658.
653 Genton, BJ, Kotanen, PM, Cheptou, PO, Adolphe, C and Shykoff, JA (2005). Enemy release but no
654 evolutionary loss of defence in a plant invasion: An inter-continental reciprocal transplant
655 experiment. *Oecologia*, **146**, 404-414.
656 Gerber, E, Schaffner, U, Gassmann, A, Hinz, H, Seier, M and MÜLLER-SCHÄRER, H (2011).
657 Prospects for biological control of *ambrosia artemisiifolia* in europe: Learning from the past.
658 *Weed Research*, **51**, 559-573.
659 Gladieux, P, Giraud, T, Kiss, L, Genton, BJ, Jonot, O and Shykoff, JA (2010). Distinct invasion
660 sources of common ragweed (*ambrosia artemisiifolia*) in eastern and western europe.
661 *Biological Invasions*, **13**, 933-944.
662 Grime, JP (1977). Evidence for the existence of three primary strategies in plants and its relevance to
663 ecological and evolutionary theory. *The American Naturalist*, **111**, 1169-1194.
664 Gu, X, Siemann, E, Zhu, L, Gao, S, Wang, Y and Ding, J (2014). Invasive plant population and
665 herbivore identity affect latex induction. *Ecological Entomology*, **39**, 1-9.

666 Hahn, PG and Maron, JL (2016). A framework for predicting intraspecific variation in plant defense.
667 *Trends in Ecology & Evolution*, **31**, 646-656.
668 Hauser, M-T (2014). Molecular basis of natural variation and environmental control of trichome
669 patterning. *Frontiers in Plant Science*, **5**, 320.
670 He, W-M, Thelen, GC, Ridenour, WM and Callaway, RM (2010). Is there a risk to living large? Large
671 size correlates with reduced growth when stressed for knapweed populations. *Biological*
672 *Invasions*, **12**, 3591-3598.
673 Heredia, JB and Cisneros-Zevallos, L (2009). The effect of exogenous ethylene and methyl jasmonate
674 on pal activity, phenolic profiles and antioxidant capacity of carrots (*daucus carota*) under
675 different wounding intensities. *Postharvest Biology and Technology*, **51**, 242-249.
676 Hodgins, KA, Bock, DG and Rieseberg, L (2018). Trait evolution in invasive species. *Annual Plant*
677 *Reviews online*, **1**, 1-37.
678 Hodgins, KA and Rieseberg, L (2011). Genetic differentiation in life-history traits of introduced and
679 native common ragweed (*ambrosia artemisiifolia*) populations. *Journal of Evolutionary*
680 *Biology*, **24**, 2731-2749.
681 Ito, K and Sakai, S (2009). Optimal defense strategy against herbivory in plants: Conditions selecting
682 for induced defense, constitutive defense, and no-defense. *Journal of Theoretical Biology*, **260**,
683 453-459.
684 Jordan, CY, Ally, D and Hodgins, KA (2015). When can stress facilitate divergence by altering time
685 to flowering? *Ecology and evolution*, **5**, 5962-5973.
686 Joshi, J and Vrieling, K (2005). The enemy release and eica hypothesis revisited: Incorporating the
687 fundamental difference between specialist and generalist herbivores. *Ecology Letters*, **8**, 704-
688 714.
689 Katabuchi, M (2015). Leafarea: An r package for rapid digital image analysis of leaf area. *Ecological*
690 *Research*, **30**, 1073-1077.
691 Kazinczi, G, Béres, I, Novák, R, Bíró, K and Pathy, Z (2008). Common ragweed (*ambrosia*
692 *artemisiifolia*): A review with special regards to the results in hungary. I. Taxonomy, origin
693 and distribution, morphology, life cycle and reproduction strategy. *Herbologia*, **9**, 55-91.
694 Keinänen, M, Oldham, NJ and Baldwin, IT (2001). Rapid hplc screening of jasmonate-induced
695 increases in tobacco alkaloids, phenolics, and diterpene glycosides in *nicotiana attenuata*.
696 *Journal of agricultural and food chemistry*, **49**, 3553-3558.
697 Kessler, A and Baldwin, IT (2002). Plant responses to insect herbivory: The emerging molecular
698 analysis. *Annual Review of Plant Biology*, **53**, 299-328.

Koricheva, J, Nykänen, H and Gianoli, E (2004). Meta-analysis of trade-offs among plant antiherbivore defenses: Are plants jacks-of-all-trades, masters of all? *The American Naturalist*, **163**, E64-E75.

Krishnamoorthy, K and Lee, M (2014). Improved tests for the equality of normal coefficients of variation. *Computational Statistics*, **29**, 215-232.

Kumschick, S, Hufbauer, RA, Alba, C and Blumenthal, DM (2013). Evolution of fast-growing and more resistant phenotypes in introduced common mullein (*verbascum thapsus*). *Journal of Ecology*, **101**, 378-387.

Lachmuth, S, Durka, W and Schurr, FM (2011). Differentiation of reproductive and competitive ability in the invaded range of *senecio inaequidens*: The role of genetic allee effects, adaptive and nonadaptive evolution. *New Phytologist*, **192**, 529-541.

Lande, R (2015). Evolution of phenotypic plasticity in colonizing species. *Molecular Ecology*, **24**, 2038-2045.

Lee, CE (2002). Evolutionary genetics of invasive species. *Trends in Ecology and Evolution*, **17**, 386-391.

Lee, J, Vogt, T, Schmidt, J, Parthier, B and Löbler, M (1997). Methyljasmonate-induced accumulation of coumaroyl conjugates in barley leaf segments. *Phytochemistry*, **44**, 589-592.

Li, Z-H, Wang, Q, Ruan, X, Pan, C-D and Jiang, D-A (2010). Phenolics and plant allelopathy. *Molecules*, **15**, 8933-8952.

Lommen, STE, Hallmann, CA, Jongejans, E, Chauvel, B, Leitsch-Vitalos, M, Aleksanyan, A, Tóth, P, Preda, C, Šćepanović, M, Onen, H, Tokarska-Guzik, B, Anastasiu, P, Dorner, Z, Fenesi, A, Karrer, G, Nagy, K, Pinke, G, Tiborcz, V, Zagyvai, G, Zalai, M, Kazinczi, G, Leskovšek, R, Stešević, D, Fried, G, Kalatozishvili, L, Lemke, A and Müller-Schärer, H (2017). Explaining variability in the production of seed and allergenic pollen by invasive *ambrosia artemisiifolia* across europe. *Biological Invasions*, **20**, 1475-1491.

Macel, M, Dostálek, T, Esch, S, Bucharová, A, van Dam, NM, Tielbörger, K, Verhoeven, KJ and Münzbergová, Z (2017). Evolutionary responses to climate change in a range expanding plant. *Oecologia*, **184**, 543-554.

Maron, JL and Vilà, M (2001). When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos*, **95**, 361-373.

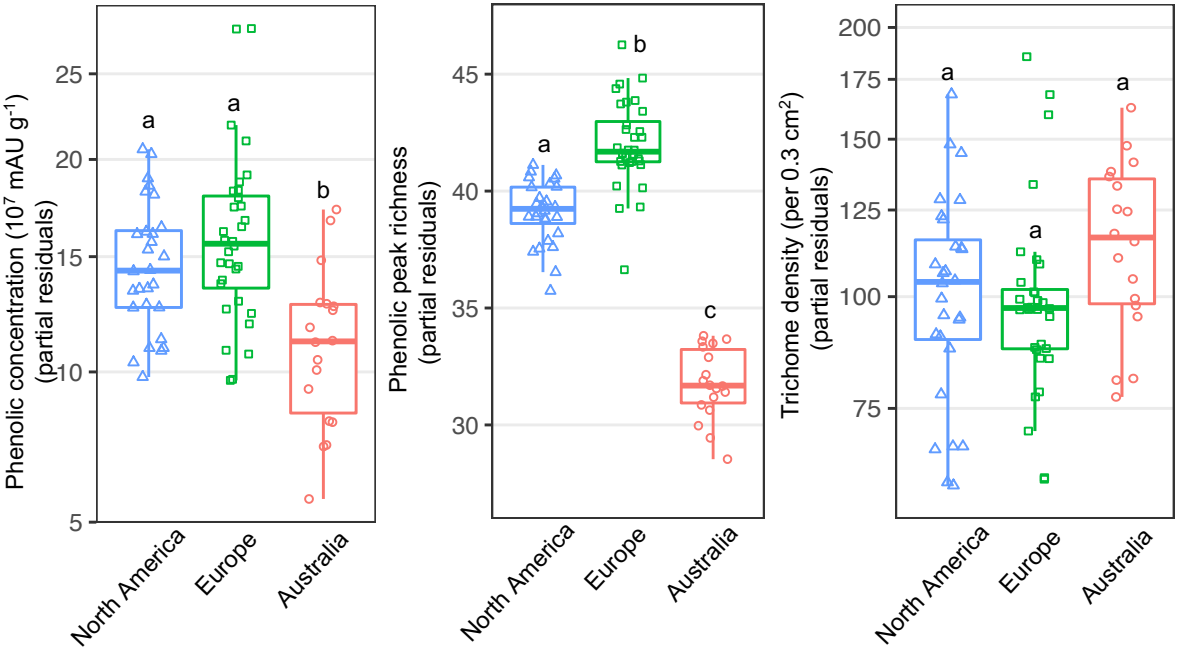
Marwick, B and Krishnamoorth, K (2018). Cvequality: Tests for the equality of coefficients of variation from multiple groups**:** R package.

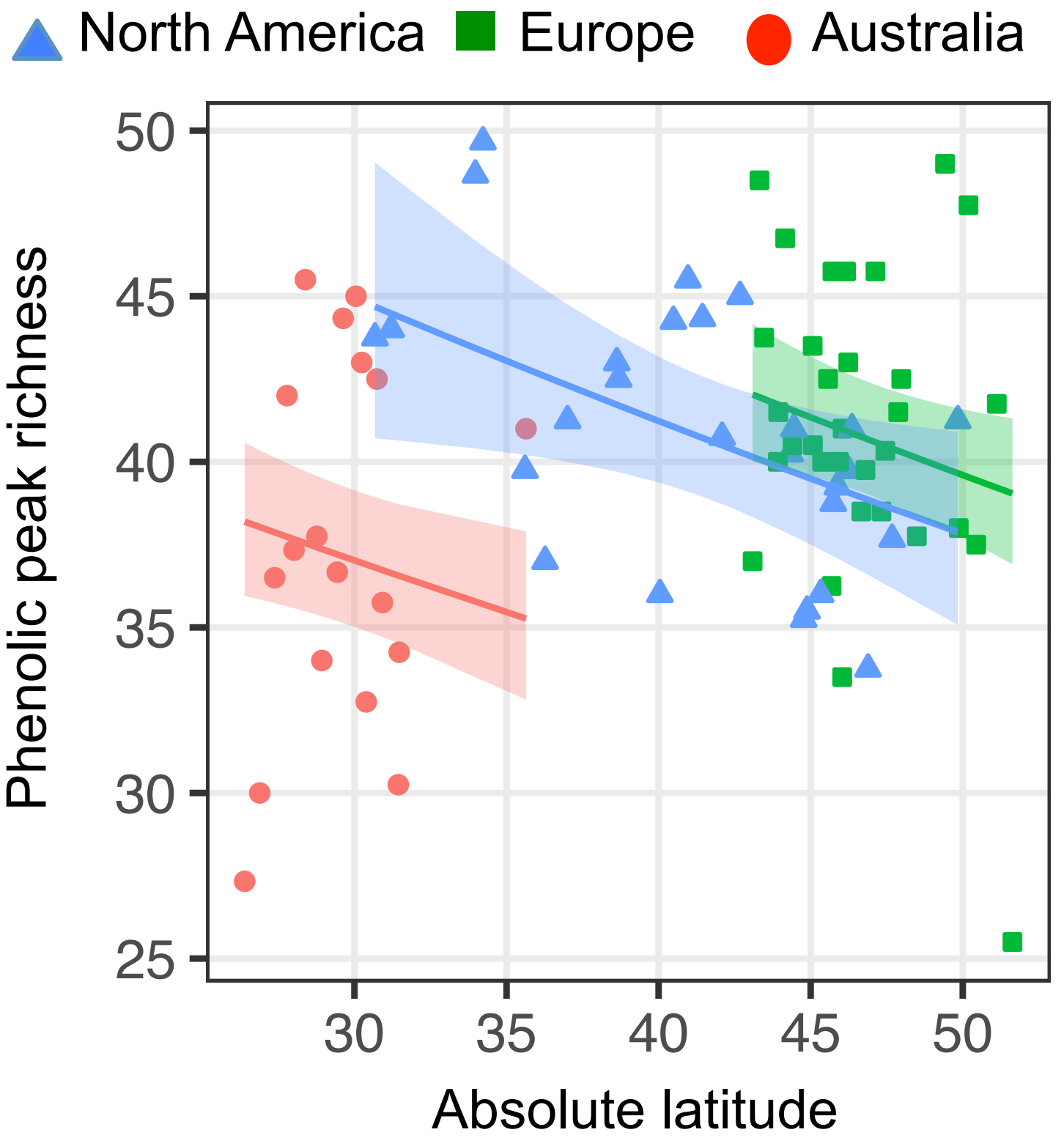
Mole, S (1994). Trade-offs and constraints in plant-herbivore defense theory: A life-history perspective. *Oikos*, 3-12.

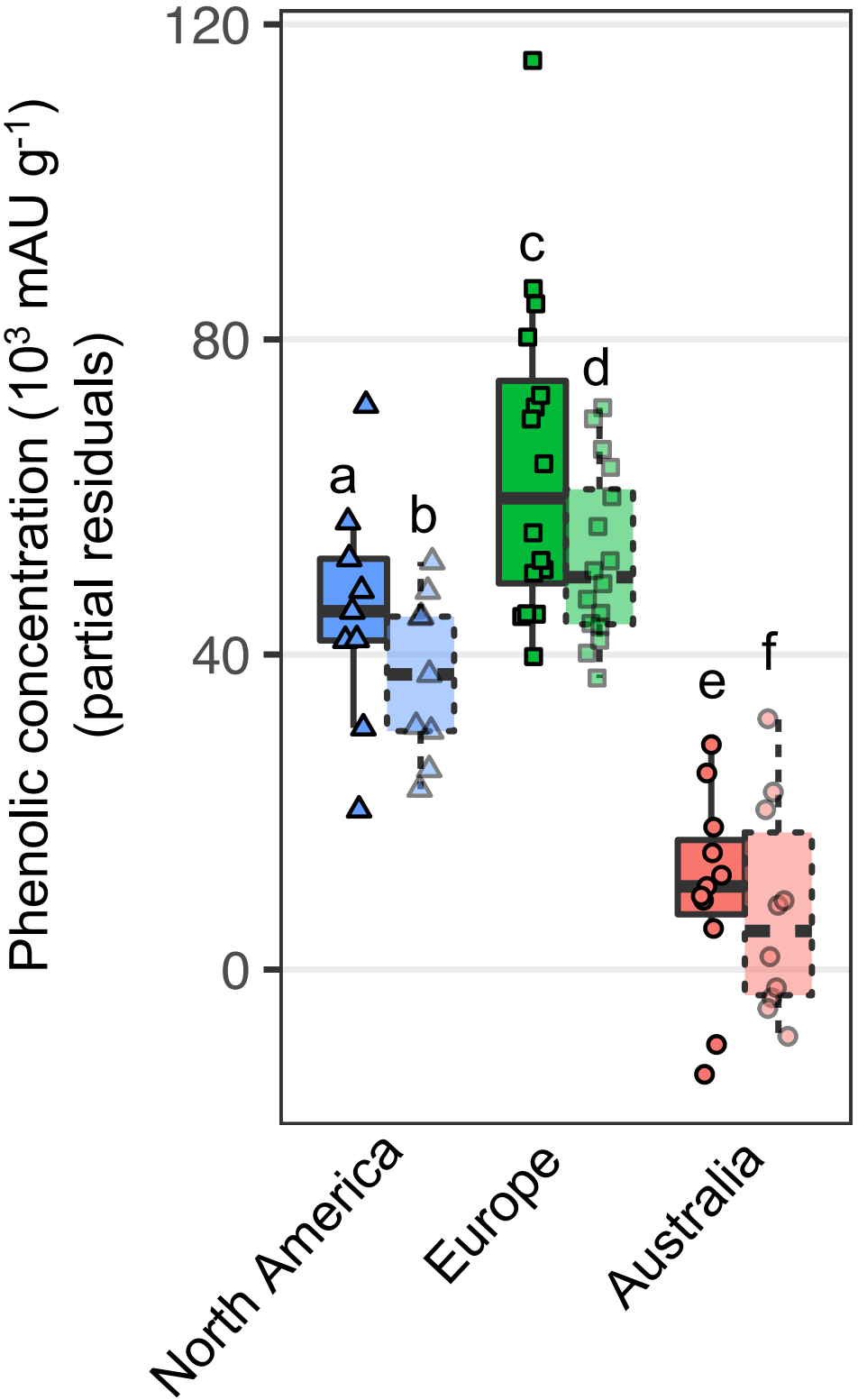
733 Moles, AT, Bonser, SP, Poore, AG, Wallis, IR and Foley, WJ (2011a). Assessing the evidence for
734 latitudinal gradients in plant defence and herbivory. *Functional Ecology*, **25**, 380-388.
735 Moles, AT, Wallis, IR, Foley, WJ, Warton, DI, Stegen, JC, Bisigato, AJ, Cella-Pizarro, L, Clark, CJ,
736 Cohen, PS and Cornwell, WK (2011b). Putting plant resistance traits on the map: A test of the
737 idea that plants are better defended at lower latitudes. *New Phytologist*, **191**, 777-788.
738 Moreira, X, Mooney, KA, Rasmann, S, Petry, WK, Carrillo-Gavilán, A, Zas, R and Sampedro, L
739 (2014). Trade-offs between constitutive and induced defences drive geographical and climatic
740 clines in pine chemical defences. *Ecology Letters*, **17**, 537-546.
741 Morris, WF, Traw, MB and Bergelson, J (2006). On testing for a tradeoff between constitutive and
742 induced resistance. *Oikos*, **112**, 102-110.
743 Müller-Schärer, H, Schaffner, U and Steinger, T (2004). Evolution in invasive plants: Implications for
744 biological control. *Trends in Ecology & Evolution*, **19**, 417-422.
745 Müller-Schärer, H, Lommen, ST, Rossinelli, M, Bonini, M, Boriani, M, Bosio, G and Schaffner, U
746 (2014). *Ophraella communa*, the ragweed leaf beetle, has successfully landed in europe:
747 Fortunate coincidence or threat? *Weed research*, **54**, 109-119.
748 Neilson, EH, Goodger, JQ, Woodrow, IE and Møller, BL (2013). Plant chemical defense: At what
749 cost? *Trends in plant science*, **18**, 250-258.
750 Orians, CM and Ward, D (2010). Evolution of plant defenses in nonindigenous environments. *Annual*
751 *Review of Entomology*, **55**, 439-459.
752 Orrock, JL, Sih, A, Ferrari, MC, Karban, R, Preisser, EL, Sheriff, MJ and Thaler, JS (2015). Error
753 management in plant allocation to herbivore defense. *Trends in Ecology & Evolution*, **30**, 441-
754 445.
755 Oswalt, ML and Marshall, GD (2008). Ragweed as an example of worldwide allergen expansion.
756 *Allergy, Asthma and Clinical Immunology*, **4**, 130-135.
757 Palmer, B and McFadyen, RE (2012). *Ambrosia artemisiifolia* l. - annual ragweed. Biological control
758 of weeds in australia. M. H. Julien, R. E. McFadyen and J. Cullen. Collingwood, CSIRO**:** 52-
759 59.
760 Parker, JD, Torchin, ME, Hufbauer, RA, Lemoine, NP, Alba, C, Blumenthal, DM, Bossdorf, O, Byers,
761 JE, Dunn, AM and Heckman, RW (2013). Do invasive species perform better in their new
762 ranges? *Ecology*, **94**, 985-994.
763 Prentis, PJ, Wilson, JR, Dormontt, EE, Richardson, DM and Lowe, AJ (2008). Adaptive evolution in
764 invasive species. *Trends in Plant Sciences*, **13**, 288-294.
765 R Core Team (2018). R: A language and environment for statistical computing (v3.5.1 "feather
766 spray"). Vienna, Austria, The R Foundation for Statistical Computing.

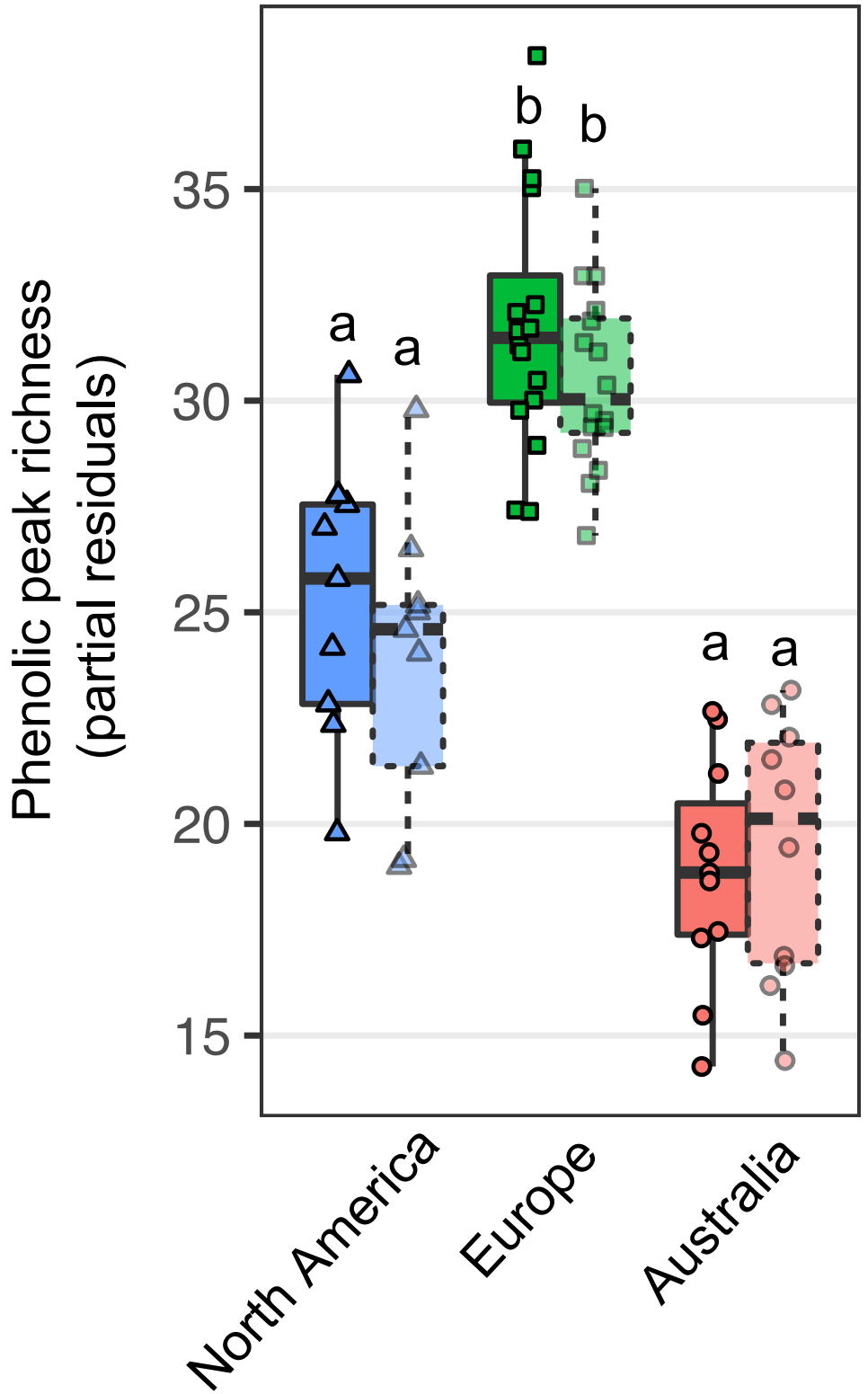
767 Rasmann, S and Agrawal, AA (2011). Latitudinal patterns in plant defense: Evolution of cardenolides,
768 their toxicity and induction following herbivory. *Ecology Letters*, **14**, 476-483.
769 Rasmann, S, Agrawal, AA, Cook, SC and Erwin, AC (2009). Cardenolides, induced responses, and
770 interactions between above-and belowground herbivores of milkweed (*asclepias spp.*).
771 *Ecology*, **90**, 2393-2404.
772 Ricciardi, A (2007). Are modern biological invasions an unprecedented form of global change?
773 *Conservation Biology*, **21**, 329-336.
774 Rius, M and Darling, JA (2014). How important is intraspecific genetic admixture to the success of
775 colonising populations? *Trends in Ecology & Evolution*, **29**, 233-242.
776 Sax, DF and Brown, JH (2000). The paradox of invasion. *Global Ecology and Biogeography*, **9**, 363-
777 371.
778 Scheiner, SM (2001). Multiple response variables and multi-species interactions. Design and analysis
779 of ecological experiments. S. M. Scheiner and J. Gurevitch. New York, Chapman & Hall**:** 99-
780 133.
781 Schrieber, K, Wolf, S, Wypior, C, Höhlig, D, Hensen, I and Lachmuth, S (2017). Adaptive and non-
782 adaptive evolution of trait means and genetic trait correlations for herbivory resistance and
783 performance in an invasive plant. *Oikos*, **126**, 572-582.
784 Sun, Y, Brönnimann, O, Roderick, GK, Poltavsky, A, Lommen, ST and Müller-Schärer, H (2017).
785 Climatic suitability ranking of biological control candidates: A biogeographic approach for
786 ragweed management in europe. *Ecosphere*, **8**, e01731.
787 Taramarcaz, P, Lambelet, C, Clot, B, Keimer, C and Hauser, C (2005). Ragweed (*ambrosia*)
788 progression and its health risks: Will switzerland resist this invasion? *Swiss Medical Weekly*,
789 **135**, 538-548.
790 Thébaud and Simberloff (2001). Are plants really larger in their introduced ranges? *The American*
791 *Naturalist*, **157**, 231-236.
792 Throop, HL (2005). Nitrogen deposition and herbivory affect biomass production and allocation in an
793 annual plant. *Oikos*, **111**, 91-100.
794 Tian, D, Tooker, J, Peiffer, M, Chung, SH and Felton, GW (2012). Role of trichomes in defense against
795 herbivores: Comparison of herbivore response to woolly and hairless trichome mutants in
796 tomato (*solanum lycopersicum*). *Planta*, **236**, 1053-1066.
797 Turner, KG, Fréville, H and Rieseberg, LH (2015). Adaptive plasticity and niche expansion in an
798 invasive thistle. *Ecology and Evolution*, **5**, 3183-3197.
799 Turner, KG, Hufbauer, RA and Rieseberg, LH (2014). Rapid evolution of an invasive weed. *New*
800 *Phytologist*, **202**, 309-321.

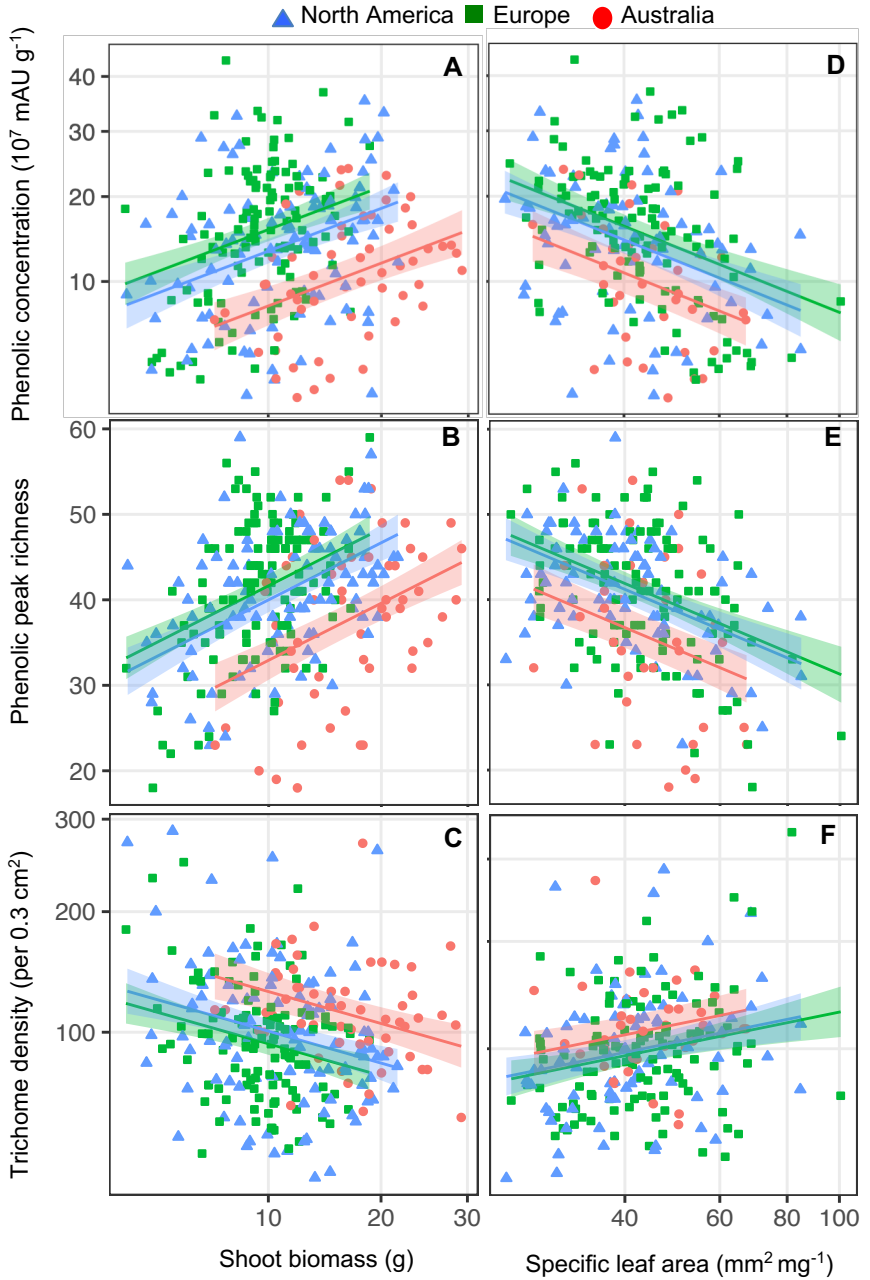
801 Uesugi, A, Connallon, T, Kessler, A and Monro, K (2017). Relaxation of herbivore-mediated selection
802 drives the evolution of genetic covariances between plant competitive and defense traits.
803 *Evolution*, **71**, 1700-1709.
804 Uesugi, A and Kessler, A (2016). Herbivore release drives parallel patterns of evolutionary divergence
805 in invasive plant phenotypes. *Journal of Ecology*, **104**, 876-886.
806 van Boheemen, LA, Atwater, DZ and Hodgins, KA (2018). Rapid and repeated local adaptation to
807 climate in an invasive plant. *New Phytologist*, **222**, 1, doi: https://doi.org/10.1101/420752.
808 van Boheemen, LA, Lombaert, E, Nurkowski, KA, Gauffre, B, Rieseberg, LH and Hodgins, KA
809 (2017). Multiple introductions, admixture and bridgehead invasion characterize the
810 introduction history of *ambrosia artemisiifolia* in europe and australia. *Molecular Ecology*, **26**,
811 5421-5434.
812 Van Noordwijk, AJ and de Jong, G (1986). Acquisition and allocation of resources: Their influence
813 on variation in life history tactics. *The American Naturalist*, **128**, 137-142.
814 Wang, Y, Carrillo, J, Siemann, E, Wheeler, GS, Zhu, L, Gu, X and Ding, J (2013). Specificity of
815 extrafloral nectar induction by herbivores differs among native and invasive populations of
816 tallow tree. *Annals of Botany*, **112**, 751-756.
817 Wang, Y, Siemann, E, Wheeler, GS, Zhu, L, Gu, X and Ding, J (2012). Genetic variation in anti-
818 herbivore chemical defences in an invasive plant. *Journal of Ecology*, **100**, 894-904.
819 War, AR, Kumar Taggar, G, Hussain, B, Sachdeva Taggar, M, Nair, RM and Sharma, HC (2018).
820 Plant defense against herbivory and insect adaptations. *AoB Plants*.
821 War, AR, Paulraj, MG, Ahmad, T, Buhroo, AA, Hussain, B, Ignacimuthu, S and Sharma, HC (2012).
822 Mechanisms of plant defense against insect herbivores. *Plant signaling & behavior*, **7**, 1306-
823 1320.
824 WHO, WHO (1998). Global solar uv index. Global Solar UV Index.
825 Willemsen, RW (1975). Dormancy and germination of common ragweed seeds in the field. *American*
826 *Journal of Botany*, **62**, 639-643.
827 Woods, EC, Hastings, AP, Turley, NE, Heard, SB and Agrawal, AA (2012). Adaptive geographical
828 clines in the growth and defense of a native plant. *Ecological Monographs*, **82**, 149-168.
829 Zandt, PAV (2007). Plant defense, growth, and habitat: A comparative assessment of constitutive and
830 induced resistance. *Ecology*, **88**, 1984-1993.
831 Züst, T and Agrawal, AA (2017). Trade-offs between plant growth and defense against insect
832 herbivory: An emerging mechanistic synthesis. *Annual Review of Plant Biology*, **68**, 513-534.
833

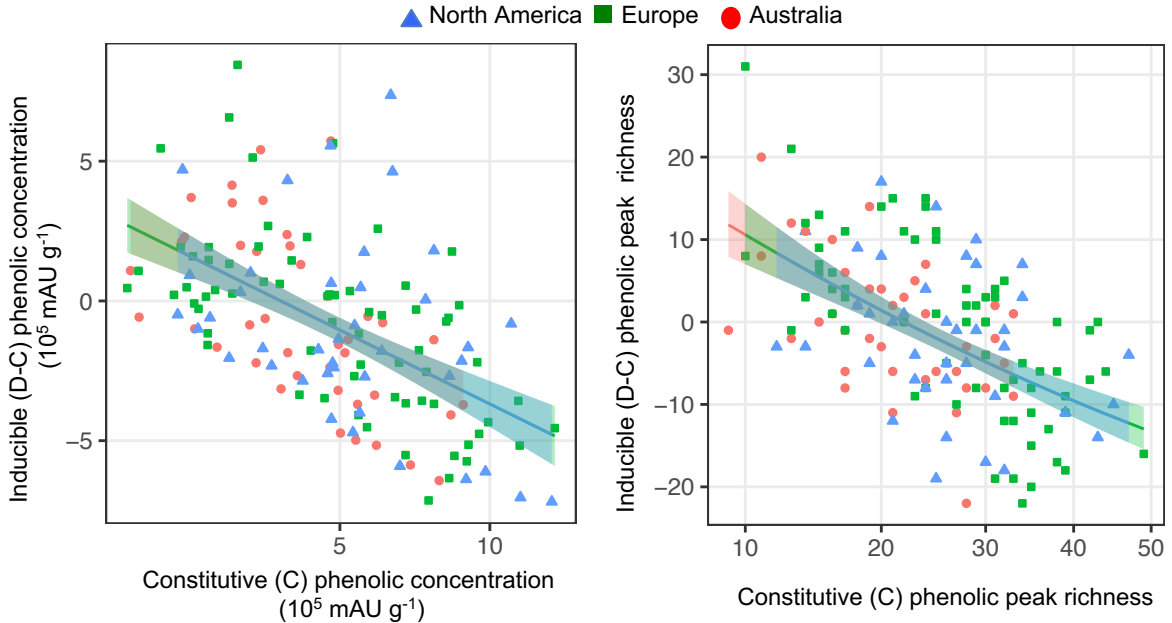

Phenolic concentration (10^7 mAU g^{-1}) (partial residuals)
25
20
15
10
5
a
a
b
North America
Europe
Australia
Phenolic peak richness (partial residuals)
45
40
35
30
a
b
c
North America
Europe
Australia
Trichome density (per 0.3 cm^2) (partial residuals)
200
175
150
125
100
75
a
a
a
North America
Europe
Australia


North America
Europe
Australia
Phenolic peak richness
50
45
40
35
30
25
30
35
40
45
50
Absolute latitude


Phenolic concentration (10^3 mAU g^{-1})
(partial residuals)
120
80
40
0
a
b
c
d
e
f
North America
Europe
Australia


Phenolic peak richness
(partial residuals)
35
30
25
20
15
a
a
b
b
a
a
North America
Europe
Australia


North America
Europe
Australia
A
B
C
D
E
F
Phenolic concentration (10^7 mAU g^{-1})
Phenolic peak richness
Trichome density (per 0.3 cm^2)
Shoot biomass (g)
Specific leaf area (mm^2 mg^{-1})
10
20
30
40
50
60
100
200
300
40
60
80
100


North America
Europe
Australia
Inducible (D-C) phenolic concentration
(10^5 mAU g^-1)
5
0
−5
5
10
Constitutive (C) phenolic concentration
(10^5 mAU g^-1)
Inducible (D-C) phenolic peak richness
30
20
10
0
−10
−20
10
20
30
40
50
Constitutive (C) phenolic peak richness