Received: 20 April 2023 | Accepted: 24 October 2023

DOI: 10.1002/ajb2.16260

American Journal of Botany

RESEARCH ARTICLE

Plant physical defenses contribute to a latitudinal gradient in resistance to insect herbivory within a widespread perennial grass

Kevin C. Headrick | **Thomas E. Juenger** | **Robert W. Heckman**

Department of Integrative Biology, University of Texas at Austin, Austin, TX 78712, USA

Correspondence

Robert W. Heckman, Department of Integrative Biology, University of Texas at Austin, Austin, TX 78712, USA; U.S. Department of Agriculture Forest Service, Rocky Mountain Research Station, Cedar City, UT 84721, USA.
Email: robert.heckman@usda.gov

Present addresses

Kevin C. Headrick, Department of Biology, Tufts University, Medford, MA 02155, USA.

Robert W. Heckman, U.S. Department of Agriculture Forest Service, Cedar City, UT 84721, USA.

Abstract

Premise: Herbivore pressure can vary across the range of a species, resulting in different defensive strategies. If herbivory is greater at lower latitudes, plants may be better defended there, potentially driving a latitudinal gradient in defense. However, relationships that manifest across the entire range of a species may be confounded by differences within genetic subpopulations, which may obscure the drivers of these latitudinal gradients.

Methods: We grew plants of the widespread perennial grass *Panicum virgatum* in a common garden that included genotypes from three genetic subpopulations spanning an 18.5° latitudinal gradient. We then assessed defensive strategies of these plants by measuring two physical resistance traits—leaf mass per area (LMA) and leaf ash, a proxy for silica—and multiple measures of herbivory by caterpillars of the generalist herbivore fall armyworm (*Spodoptera frugiperda*).

Results: Across all genetic subpopulations, low-latitude plants experienced less herbivory than high-latitude plants. Within genetic subpopulations, however, this relationship was inconsistent—the most widely distributed and phenotypically variable subpopulation (Atlantic) exhibited more consistent latitudinal trends than either of the other two subpopulations. The two physical resistance traits, LMA and leaf ash, were both highly heritable and positively associated with resistance to different measures of herbivory across all subpopulations, indicating their importance in defense against herbivores. Again, however, these relationships were inconsistent within subpopulations.

Conclusions: Defensive gradients that occur across the entire species range may not arise within localized subpopulations. Thus, identifying the drivers of latitudinal gradients in herbivory defense may depend on adequately sampling the diversity within a species.

KEYWORDS

common garden, defense investment, genome-wide association, grass evolution, latitudinal herbivory defense hypothesis, Poaceae, quantitative genetics, switchgrass

The level of herbivory on a plant varies spatially and temporally, leading to differences in plant defensive strategies (Mithöfer and Boland, 2012; Farmer, 2014). One important determinant of plant defensive strategies is the latitude from which plant populations originate. At lower latitudes, the likelihood of interacting with herbivores tends to be higher, which can lead to greater selection pressure for increased resistance to herbivory (Schemske et al., 2009; Anstett et al., 2016). Similarly, growing seasons at lower latitudes are often longer, which may promote greater leaf longevity (Kikuzawa et al., 2013). Because of their high construction costs, long-lived leaves tend to be well defended against herbivores, pathogens, and abiotic damage. In particular, physical defenses against herbivory may be important (Moles et al., 2011b). Because herbivory often exerts strong selective pressure on plant populations, genetic links between resistance traits and herbivory may evolve (Anderson and Mitchell-Olds, 2011). However, because herbivory pressure can vary spatially, these relationships may vary among geographically separated populations within species. Thus, understanding the role of latitude in plant defensive strategies may require identifying the genetics underlying plant resistance to herbivory.

The latitudinal herbivory defense hypothesis (LHDH) states that a species and populations of a species from lower latitudes will be better defended against herbivores than species or populations from higher latitudes (Anstett et al., 2016). Support for the LHDH has been found in many studies, including a study in *Asclepias* species (Rasmann and Agrawal, 2011). Within species, support for the LHDH has been inconsistent (e.g., Kooyers et al., 2017; Baskett and Schemske, 2018). This inconsistency may be related to differences in the environmental conditions across the species' range. For instance, in *Artemisia californica*, water availability increases with increasing latitude, which may explain why the LHDH holds in this species (Croy et al., 2022). On the other hand, in *Mimulus guttatus*, the prevailing resource gradient does not track the latitudinal gradient, leading to large differences in selective pressure throughout the species' range and the failure of the LHDH (Kooyers et al., 2017). Another source of inconsistency may be strong population structure (i.e., limited gene flow between populations). When gene flow between populations is limited and populations occupy distinct habitats, populations may evolve different defensive strategies, which may result in different levels of support for the LHDH within and across populations.

Plants are defended in myriad ways. One important type of defense is physical defense, which includes any trait that makes a plant's tissues harder to eat, break down, or digest (Coley and Barone, 1996; Moles et al., 2013). Unlike chemical defenses, which are often highly variable between species, many physical defenses are common across and within plant species (Moles et al., 2011b; Farmer, 2014). This ubiquity makes physical defenses good candidates for assessing the LHDH. Among grasses, one major physical defense is silica, which is deposited in leaves as abrasive bodies that can wear down the teeth or mandibles of chewing herbivores and can be estimated by measuring leaf ash (McNaughton et al., 1985; Moles et al., 2011b). Another important physical defense is leaf toughness, which makes leaves more difficult for herbivores to consume (Pérez-Harguindeguy et al., 2013). Leaf toughness can be estimated by measuring leaf mass per area (LMA)—the ratio of dry leaf mass to single-sided leaf area (Onoda et al., 2011). As an indication of the importance of these physical defenses, a recent large-scale community-level study showed that plant species from higher latitudes possessed higher LMA and leaf ash, which is contrary to the LHDH (Moles et al., 2011b).

It is not uncommon for relationships that hold across broad groups of species, like the LHDH, to fall apart within species (Agrawal, 2020), often because single species rarely occupy sufficiently broad gradients to reconstitute the driver of the across-species relationship. But, among widespread species exhibiting adequate phenotypic and genetic diversity, these relationships are sometimes detectable. Moreover, because evolution actively occurs within populations of species, the evolution of defensive strategies is most readily discernible within populations. Within-species studies also allow researchers to use quantitative genetic methods to better understand how defensive strategies evolve (Lynch and Walsh, 1998; Anderson and Mitchell-Olds, 2011). One way to leverage quantitative genetics is to grow plants from diverse localities in a common environment where genetic effects can be isolated from environmental effects, which can be confounded when studies are performed in situ (Anstett et al., 2015; Kooyers et al., 2017; Napier et al., 2023). In this way, it is possible to quantify the amount of genetic variation present in traits, which is necessary, but not sufficient for predictable evolution to occur (i.e., heritability; Lynch and Walsh, 1998; Mackay, 2001). An important source of covariation between functionally related traits is shared genetic architecture—when two distinct traits are encoded by the same gene or by genes in close proximity, traits are typically inherited together (i.e., genetic correlation). Genetic architecture can constrain the rate of phenotypic evolution when conflicting selective pressure acts on multiple traits (Walsh and Blows, 2009). But when selection also favors these genetically correlated traits, evolution can proceed rapidly (Lynch and Walsh, 1998; Walsh and Blows, 2009). For instance, Parker et al. (2012) quantified heritability and genetic correlations among root and shoot chemical defenses in *Oenothera biennis* to conclude that sufficient genetic variation existed in root chemical defenses to facilitate the continued evolution of resistance to herbivory.

Both heritability and genetic correlation define aggregate genetic effects, which may be the sum of offsetting genetic contributions. For instance, one genetic locus may contribute positively to the relationship between two traits, while another could contribute negatively, potentially leading to offsetting effects that would not be detectable in genetic correlations. To understand the particular genomic regions that contribute to these traits and trait combinations, genetic mapping approaches are valuable (Mauricio, 2001; Anderson et al., 2011). In particular, genome-wide association (GWA) mapping assesses the statistical relationship between single nucleotide polymorphisms (SNPs) and a trait of interest (Tibbs Cortes et al., 2021).

Given the important role of genetics in herbivory resistance (Anderson and Mitchell-Olds, 2011), discrepancies in the LHDH could be addressed well with species that occupy a broad range of habitats and have well-understood genomic architecture, as is present in the widespread C_4 grass *Panicum virgatum* L. (Poaceae). In this study, we used a diverse group of resequenced *P. virgatum* genotypes coming from three genetic subpopulations to examine latitudinal gradients in herbivory defense (Figure 1). We measured two physical resistance traits—LMA and leaf ash—hypothesized to confer resistance to herbivory by generalist fall armyworm caterpillars (*Spodoptera frugiperda* J.E. Smith) in the field and in lab-based feeding assays. We addressed five questions: (1) Does herbivory increase with latitude of origin across and within subpopulations? (2) Is there genetic variation underlying physical resistance traits and measures of herbivory? (3) Do high LMA and leaf ash reduce herbivory? (4) Are measures of herbivory positively genetically correlated with one another? (5) Which genomic regions are associated with physical resistance traits and measures of herbivory?

MATERIALS AND METHODS

Plant system

Panicum virgatum is native to North America, where it is an ecologically important component of mesic grasslands, and is a potentially economically important candidate bioenergy crop (Casler, 2012; Lovell et al., 2021). It occurs in three genetic subpopulations—Midwest, Atlantic, and Gulf—that differ in size and leaf morphology: the Midwest has small, thin leaves; the Atlantic has slightly larger, broad leaves; the Gulf has the largest, and typically thickest, leaves (Casler, 2012; Lovell et al., 2021). These subpopulations also differ in foliar nitrogen (N) content and susceptibility to foliar fungal pathogens. The Midwest ecotype, which occurs at higher latitudes, has higher N content and is substantially more susceptible to fungal disease than the Gulf ecotype, which occurs at lower latitudes (Aspinwall et al., 2013; Heckman et al., 2020; VanWallendael et al., 2020). These characteristics make *P. virgatum* an ideal system to explore the relationship between physical traits of leaves and resistance to herbivory.

The *P. virgatum* genotypes used in this study came from seed and rhizome collections from natural occurrences throughout the eastern and central United States (Figure 1; Lovell et al., 2021). These genotypes came from all three genetic subpopulations identified by Lovell et al. (2021) and spanned a latitudinal range of 18.5 degrees (26.87°–45.38° N). Of the genotypes, 335 were of known latitude of origin and 48 lacked a precise origin. Plants were acquired between 2010 and 2018. All plants were propagated at the University of Texas at Austin's Brackenridge Field Lab (Austin, Texas, USA). Specifically, all

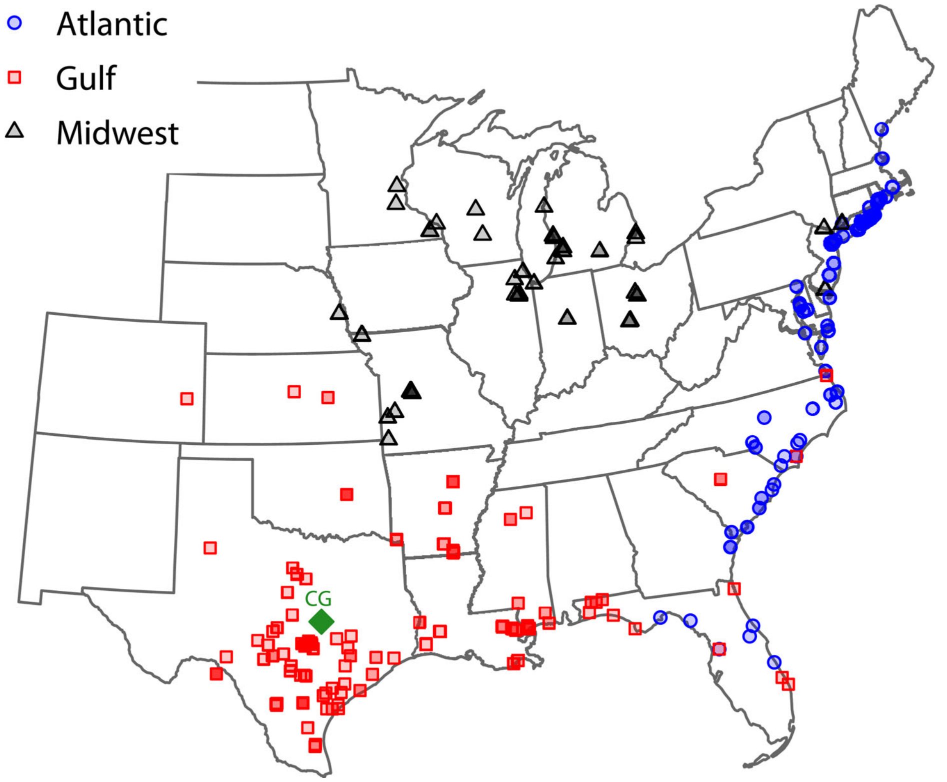

FIGURE 1 Map showing origins of *P. virgatum* genotypes from three genetic subpopulations, that have limited geographical overlap. Genotypes were propagated at the University of Texas at Austin, then planted at a common garden (CG) in Temple, Texas, United States. Points have partly transparent filling; darker shading indicates that more genotypes come from the same or nearby locations.

plants—whether collected as seed or rhizome—were grown in 18.9-L pots until they had produced enough tillers to be divided. The crown of each plant was then divided; one clone of each plant was transported to the U.S. Department of Agriculture Grassland Soil and Water Research Lab in Temple, Texas, USA and planted in May 2018. Both the site of propagation and the site of this study were in the region primarily occupied by the Gulf subpopulation. Because all propagation was clonal, the plants in this experiment were genetically identical to the original seed or rhizome. Thus, no adaptive changes in plants were possible. Plants were randomly arranged in a honeycomb pattern with 1.56-m spacing. The field was 45 × 42 m and covered in landscape fabric (Sunbelt 3.2 oz, DeWitt, Sikeston, MO, USA) to reduce competition from weeds. In total, our study comprised 398 individual plants. To prevent edge effects, we planted a row of plants of the upland *P. virgatum* cultivar Blackwell around the field.

Lovell et al. (2021) describe the methods for sequencing and annotating the *P. virgatum* genome, assigning plants to genetic subpopulations, and resequencing efforts to genotype the plants included in this study. Briefly, the *P. virgatum* genome was sequenced using a genotype of the cultivar Alamo (AP13). Then, all genotypes used in this study were resequenced using Illumina HiSeq X10 and Illumina NovaSeq 6000 paired-end sequencing (2 × 150 bp) and mapped to the genome reference (bwa-mem; Li and Durbin, 2009). After filtering for missing data and minor allele frequencies (>0.05), we retained 10.0 M SNPs to generate an additive genetic relationship matrix and to perform association mapping (described below).

Fall armyworm biology

In this study, we examined resistance to herbivory in *P. virgatum* using the generalist herbivore *Spodoptera frugiperda* (fall armyworm). We focused on *S. frugiperda* for several reasons. First, we observed extensive natural *S. frugiperda* herbivory within our experimental planting, and *S. frugiperda* has successfully fed upon *P. virgatum* in experimental studies (Dowd and Johnson, 2009; Nabity et al., 2011; Nabity et al., 2012; Dowd et al., 2013; Schuh et al., 2018). Second, they are a generalist chewing herbivore and have served as a model for generalist herbivory in numerous ecological and evolutionary studies (e.g., Travers-Martin and Müller, 2008; Moreno et al., 2009; Erb et al., 2011). As such, their biology is well known, and caterpillars are commercially available. Third, *S. frugiperda* is a species of emerging concern in agricultural systems worldwide. Recently *S. frugiperda* has spread to maize, rice, and other important crops in East Asia, causing significant damage (He et al., 2021; Sun et al., 2021). As with most lepidopterans, the larval stage of *S. frugiperda* feeds extensively on leaf tissue (Capinera, 2002). These caterpillars grow through six larval instars before pupation (Deshmukh et al., 2021).

Leaf collection

Separate leaves were collected to measure physical leaf resistance (LMA and leaf ash) and to quantify herbivory by caterpillars in lab-based feeding trials (described below). For both purposes, we selected one young, fully emerged leaf that lacked visible pathogen or herbivore damage from each of several tillers per plant. Among the large number of leaves per plant that met these criteria and were representative of the canopy as a whole, 5–10 leaves were randomly selected. Leaves were cut at their base and placed in a test tube with distilled water within 10 min. Cut leaves remained in a cool, dark location for at least 4 h to ensure that they were all adequately rehydrated.

Physical leaf resistance traits

We measured two traits hypothesized to act as physical defenses against herbivory: leaf mass per area (LMA, the ratio of dried leaf mass to fresh leaf area; Pérez-Harguindeguy et al., 2013) and leaf ash (a proxy for silica content; Moles et al., 2011). High LMA is often associated with increased resistance to herbivory (Hanley et al., 2007; Moles et al., 2011b; but see Heckman et al., 2019), while silica is particularly important for herbivory resistance in grasses (McNaughton et al., 1985; Hartley and DeGabriel, 2016). Leaf area was measured using the single-sided leaf area of five young, fully emerged leaves from each plant using a leaf area meter (LI-3100; LI-COR Biosciences, Lincoln, NE, USA). The leaves were then dried at 60°C for 48 h and weighed. Because measuring silica can be expensive and difficult, leaf ash—the inorganic compounds remaining after combustion—can be used as a proxy (Moles et al., 2011b). To calculate leaf ash, we ground dried leaves to a fine powder (Geno/Grinder 2010, Spex Sample Prep, Metuchen, NJ, USA), then 0.400 g (±0.001 g) of ground leaf tissue was combusted in a muffle furnace at 500°C for 12 h (Thermo Lindberg/Blue M Moldatherm, Thermo Scientific, Asheville, NC, USA). Leaf ash is the proportion of sample mass remaining after combustion. While leaf ash can be considered a proxy for silica content due to the high silica content of many grasses—including *P. virgatum*, where ash can be half to two-thirds silica (Lanning and Eleuterius, 1983, 1987)—ash includes many inorganic compounds, including potassium, calcium, and magnesium.

Feeding trials with laboratory caterpillars

Fall armyworm caterpillars were purchased from a commercial lab (Benzon Research, Carlisle, PA, USA) and reared at 25–27°C on a corn-based food medium with a 16-h light/8-h dark photoperiod under 340 lumen lights. These caterpillars came from a laboratory strain developed by the U.S. Department of Agriculture in Mississippi and have been reared to remain genetically consistent over generations. There is moderate genetic differentiation between this laboratory strain and field populations in the United States (Schlum et al., 2021). Once the caterpillars reached the third–fourth instar, we placed each caterpillar into an empty Petri dish with a small droplet of water. We sealed the Petri dishes with parafilm and returned them to the same light and temperature conditions.

After the caterpillars were starved for 18–24 h, we began nonchoice feeding trials. First, each caterpillar was weighed, and only individuals with a starved mass between 0.08 and 0.16 g were used. Second, to each dish with a caterpillar, we added several drops of water and 40–50 cm^2 of leaf tissue (2–6 leaves per plant) for which we recorded the exact area added, then sealed the dishes with parafilm and returned them to the climate-controlled room for ~48 h. No caterpillars consumed all the leaves during the feeding trial. Third, at the end of the 2-d feeding trial, we reweighed each caterpillar and placed the unconsumed leaf tissue in a resealable plastic bag with a damp paper towel to rehydrate in a refrigerator for 3 days. We measured the area of the unconsumed leaf tissue with a leaf area meter (LI-3100). Any caterpillar that pupated within 2 days of the feeding trial was removed from further analysis.

We quantified two measures of herbivory in the lab—leaf mass consumed and change in caterpillar mass. Leaf mass consumed was the product of LMA and leaf area consumed; change in caterpillar mass was the ratio of mass gained (or lost) during the feeding trial to initial mass. Because the caterpillars in this trial were from a laboratory strain exhibiting limited genetic variation (Schlum et al., 2021), change in caterpillar mass was driven, at least in part, by the plant tissue they consumed. Thus, we consider change in caterpillar mass to be a plant trait.

Field herbivory

In this genetic mapping population of *P. virgatum*, we measured natural herbivory in October 2018 that occurred as a result of an invasion of *S. frugiperda* in the preceding weeks. We estimated the percentage of canopy defoliation on each plant in 2% intervals from 1% to 5% defoliation, then by 5% intervals from 5–99% defoliation (i.e., 1%, 3%, 5%, 10%, 15%, etc.). The field and lab portions of this study used the same plants. Thus, if field herbivory, which occurred in fall 2018, induced an increase in chemical or physical defenses, the increase could have impacted the leaves used in the subsequent lab feeding trials.

Data analyses

All statistical tests were performed using R version 4.3.1 (R Core Team, 2023). We first tested the LHDH by evaluating the effect of mean-centered latitude of genotype origin (a continuous variable for each genotype) on each measure of herbivory (leaf mass consumed, change in caterpillar mass, field damage) across all genotypes and within genetic subpopulations (a grouping variable with three levels: Atlantic, Gulf, Midwest). We further examined the LHDH by characterizing differences in physical resistance traits (LMA, leaf ash) and herbivory measures among the three geographically separated genetic subpopulations. We then evaluated the effects of mean-centered and variance-standardized physical resistance traits on each measure of herbivory. We used the lm() function for these analyses. Each model except field damage included several potentially confounding covariates: the identity of the leaf collector, initial caterpillar mass and the square of the initial caterpillar mass (to control for linear and nonlinear effects of caterpillar mass on herbivory), and an offset for the length of the feeding trial, which varied between 47.76 and 48.79 h. We performed post hoc comparisons of the three genetic subpopulations using the emmeans package (Lenth, 2022).

To estimate narrow-sense heritability (h^2) and genetic correlations between each pair of traits (r_g), we used a linear mixed modeling approach (i.e., animal model; Wilson et al., 2010) with the sommer package (Covarrubias-Pazaran, 2016). Each model included an additive genetic relationship matrix as a random effect, which was calculated using the VanRaden method (VanRaden, 2008; Lovell et al., 2021). Specifically, for each pair of plants, the similarity at each locus (here, SNPs) was calculated, weighted by the minor allele frequency of the locus, and summed across loci. Based on this method, relatedness between individuals that share a rare variant will be higher than relatedness between individuals that share a common variant. Here, incorporating a genetic relationship matrix into the model allowed us to statistically account for an important source of non-independence—additive genetic relatedness among genotypes—which can be used to partition variance and covariance in traits between genetic and other sources (Lynch and Walsh, 1998; Wilson et al., 2010). In univariate models, narrow-sense heritability was the ratio of additive genetic variance to total phenotypic variance. In multivariate models, the genetic correlation between each pair of traits was the ratio of genetic covariance between traits to the square root of the product of their additive genetic variances (Wilson et al., 2010). We calculated the significance of genetic correlations with a likelihood ratio test comparing a model in which the genetic covariance between the traits was freely estimated to a model in which the genetic covariance was fixed at 0. For both h^2 and r_g, standard errors were calculated using the delta method (vpredict() function in package sommer).

We performed GWA mapping for all traits (switchgrassGWAS package; Lovell et al., 2021). Each GWA model included 10.0 M biallelic SNPs and accounted for population structure by incorporating up to 10 principal components of the SNP matrix as covariates. We adjusted the *P*-values of these hypothesis tests using a 10% false discovery rate (Storey and Tibshirani, 2003). We identified candidate genes for each trait as those genes within 10 kb of a significant SNP.

Within each type of analysis, we only included plants with measured values for all responses and predictors (i.e., complete cases), but the number of plants differed across analysis types. For quantitative genetic analyses (i.e., heritability, genetic correlation, and GWA), we averaged across individuals within genotypes before analysis for the small number of genotypes that were replicated within our common garden. Similarly, 48 genotypes lacked a known latitude of origin. Thus, those genotypes were omitted from analyses examining latitude of origin, but were retained elsewhere. Moreover, field damage was measured on some genotypes that lacked other measurements. For univariate analyses of field damage, we retained all genotypes, but removed those genotypes from genetic correlations. Finally, because this study was performed in the Gulf region, the climate could differ substantially from the typical environments of Atlantic and Midwest genotypes. These differences may result in maladaptive plasticity among genotypes from the Atlantic and Midwest subpopulations, which would be difficult to isolate statistically from adaptive genetic variation using this design.

RESULTS

Does herbivory increase with latitude of origin across and within subpopulations?

The relationship between latitude of genotype origin and resistance to herbivory was inconsistent. Across all genotypes, caterpillars gained more mass when feeding on higher-latitude genotypes ($P < 0.001$; Appendix S1, Table S1A) and field damage was greater on higher-latitude genotypes ($P < 0.001$; Appendix S1, Table S1B); both results are consistent with the LHDH. However, there was no latitudinal gradient in leaf mass consumption overall ($P = 0.910$; Appendix S1, Table S1C), which is contrary to the LHDH. Within genetic subpopulations, these latitudinal trends were also inconsistent. Caterpillars gained more mass on higher-latitude genotypes within the Atlantic ($P < 0.001$) and Midwest ($P = 0.012$) subpopulations, but not in the Gulf subpopulation ($P = 0.149$; Figure 2A; Appendix S1, Table S2A). Moreover, the latitudinal trends in the Atlantic and Midwest subpopulations were significantly more positive than in the Gulf subpopulation ($P < 0.001$ and $P = 0.012$, respectively; Appendix S1, Table S3A). Higher-latitude genotypes experienced more field leaf damage in the Atlantic subpopulation ($P = 0.007$), but there was no effect of latitude on field leaf damage in the Midwest and Gulf subpopulations ($P = 0.807$ and $P = 0.160$, respectively; Figure 2B; Appendix S1, Table S2B). This latitudinal trend in the Atlantic subpopulation was significantly more positive than in the Gulf subpopulation ($P = 0.012$), but did not differ from the Midwest subpopulation ($P = 0.784$; Appendix S1, Table S3B). Unlike the other two measures of herbivory, there was no latitudinal gradient in leaf mass consumption within subpopulations ($P = 0.580$; Appendix S1, Tables S1C, S2C, S3C), which is inconsistent with the LHDH. These results indicate that the latitudinal gradients in herbivory observed here are driven by a combination of differences among subpopulations and within-subpopulation latitudinal trends.

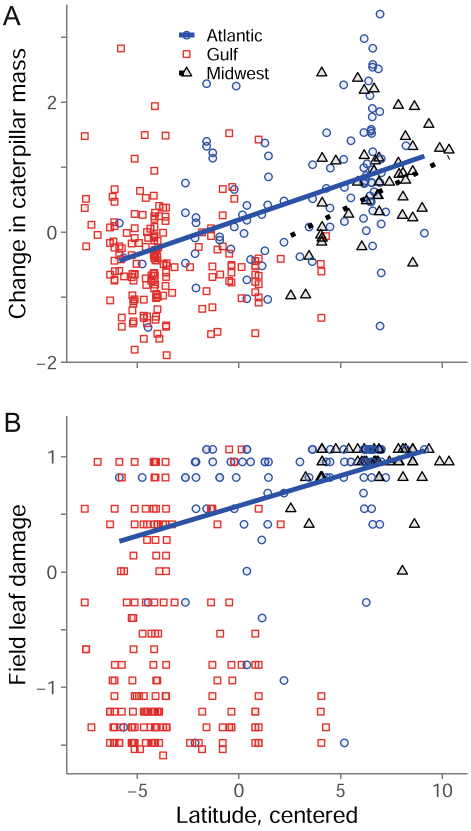

FIGURE 2 Relationship between latitude of origin and (A) change in caterpillar mass, (B) field damage separately by *P. virgatum* genetic subpopulation. Latitude of origin is mean-centered (mean = 0); measures of herbivory are mean-centered and variance-standardized (mean = 0, standard deviation = 1). Fit lines are only plotted for significant subpopulation-level effects.

Is there genetic variation underlying physical resistance traits and measures of herbivory?

Both physical resistance traits and two of the three measures of herbivory differed among genetic subpopulations (Appendix S1, Table S4). Three of these responses exhibited a similar relationship—LMA was higher and field leaf damage and change in caterpillar mass were lower in the Gulf subpopulation than in the Atlantic and Midwest subpopulations, which suggests that LMA contributes to defense against herbivory (Figure 3; Appendix S1, Table S5). Leaf ash and leaf mass consumed both exhibited a similar relationship, albeit with different degrees of statistical significance. Leaf ash was highest and leaf mass consumed was lowest in the Midwest subpopulation; leaf ash was lowest and leaf mass consumed was highest in the Atlantic subpopulation. While subpopulation differences were highly

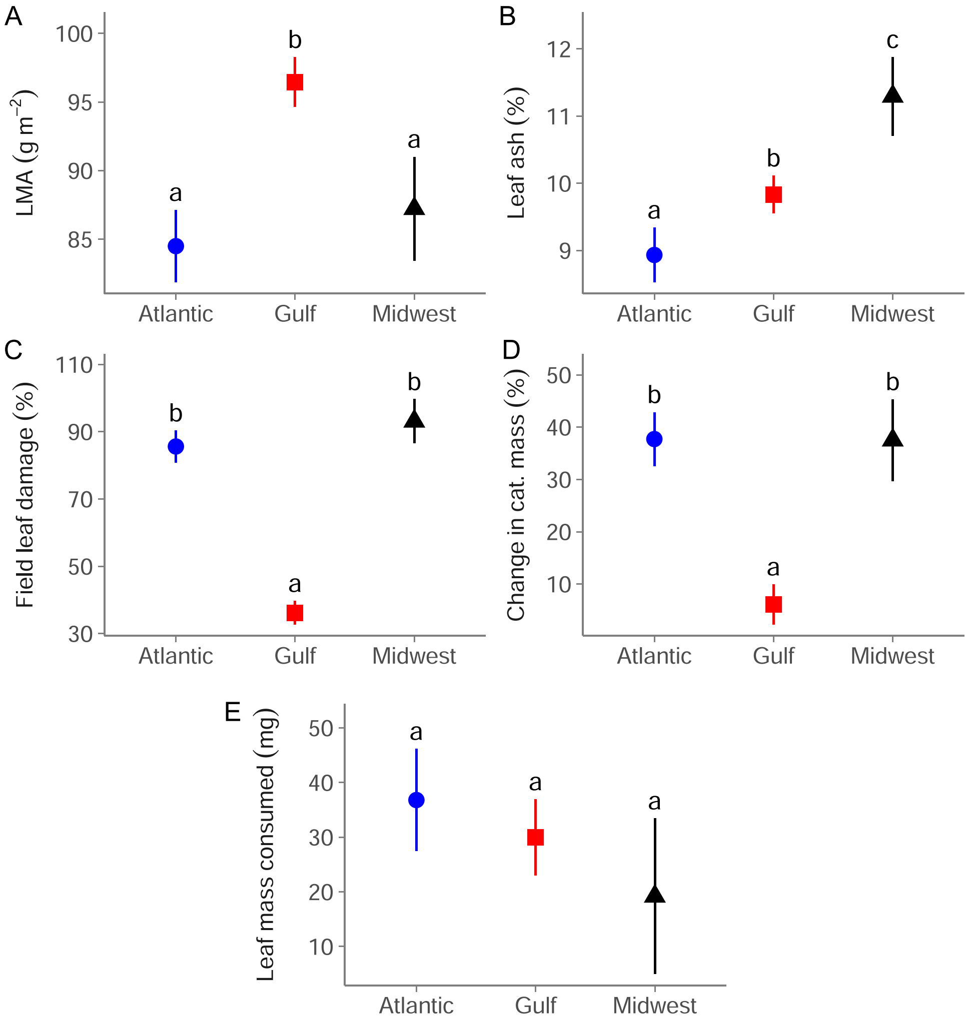

FIGURE 3 Relationship between *P. virgatum* genetic subpopulation and (A, B) leaf resistance traits and (C–E) measures of herbivory. Shared letters indicate no significant differences among genetic subpopulations. Estimates are model-derived after accounting for technical covariates. Error bars represent 95% confidence intervals.

significant in leaf ash ($P < 0.001$; Figure 3B; Appendix S1, Table S5B), in leaf mass consumed, Midwest plants experienced only marginally lower leaf mass consumption than Atlantic plants ($P = 0.074$; Figure 3E; Appendix S1, Table S5E).

Heritability also differed considerably among physical resistance traits and measures of herbivory. Among physical resistance traits, LMA was more heritable than leaf ash ($h^2 = 0.761 \pm 0.139$ and $h^2 = 0.340 \pm 0.158$, respectively), although both traits had high heritability. Among the measures of herbivory, field leaf damage ($h^2 = 0.840 \pm 0.110$) and change in caterpillar mass ($h^2 = 0.489 \pm 0.169$) were more heritable than leaf mass consumed in the lab ($h^2 = 0.054 \pm 0.061$).

Do high LMA and leaf ash reduce herbivory?

Each physical resistance trait was negatively associated with at least one measure of herbivory (Appendix S1, Table S6), indicating that these traits provide herbivore resistance. Leaf mass per area generally had a larger impact on herbivory than leaf ash, but impacts of LMA and leaf ash differed considerably among subpopulations. Caterpillars gained less mass on high-LMA plants across all genotypes ($P < 0.001$) and within the Atlantic and Midwest subpopulations ($P < 0.001$ and $P = 0.003$, respectively; Figure 4A; Appendix S1, Table S7A), but not in the Gulf subpopulation ($P = 0.980$). Similarly, field leaf damage was lower on high-LMA plants across all genotypes ($P < 0.001$), but this relationship was not significant within any subpopulations ($P > 0.10$ for all subpopulations; Figure 4B; Appendix S1, Table S7B).

Unlike LMA, leaf ash was not associated with caterpillar mass gain across all subpopulations ($P = 0.218$) or within the Gulf and Midwest subpopulations ($P = 0.204$ and $P = 0.111$, respectively; Appendix S1, Table S6). Only in the Atlantic subpopulation did caterpillars gain less mass on high-ash plants ($P = 0.004$; Figure 4C; Appendix S1, Table S7A). Moreover, leaf ash was negatively associated

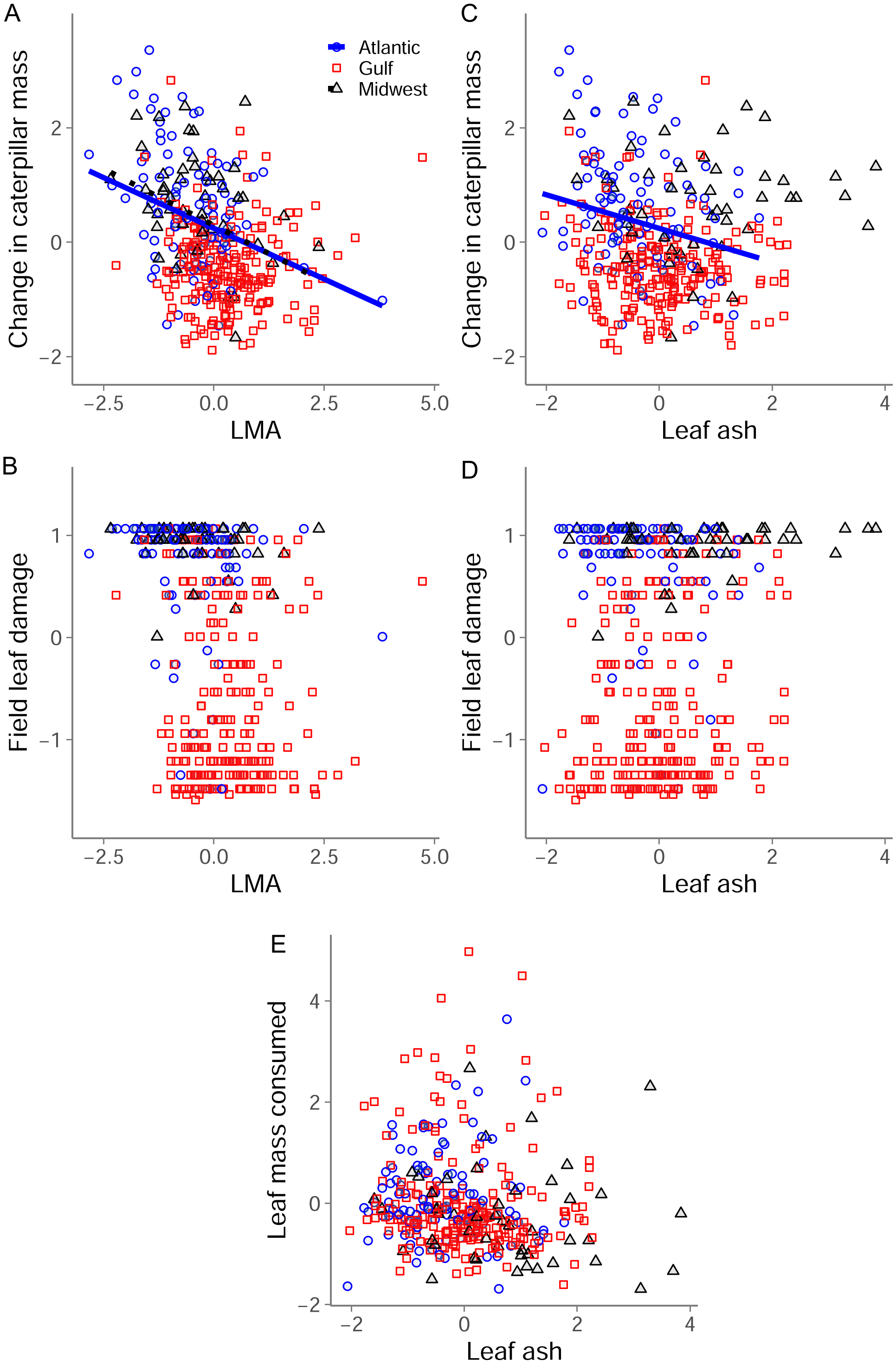

FIGURE 4 Relationship between physical leaf resistance traits (LMA, leaf ash) and three measures of herbivory separately by *P. virgatum* genetic subpopulations. All variables are mean-centered and variance-standardized (mean = 0, standard deviation = 1). Fit lines are only plotted for significant subpopulation-level effects.

with field leaf damage ($P = 0.028$), but this effect was only marginally significant within the Gulf subpopulation ($P = 0.099$) and nonsignificant within the other subpopulations ($P > 0.35$ for each subpopulation; Figure 4D; Appendix S1, Table S7B). Finally, caterpillars consumed marginally more leaf mass in the lab on low-ash plants across all genotypes ($P = 0.053$), but not within any subpopulation ($P > 0.1$ for all subpopulations; Figure 4E; Appendix S1, Table S7C).

Genetic correlations largely mirrored the phenotypic results in their direction of effect, although only a few genetic correlations were statistically significant (Table 1).

TABLE 1 Genetic correlations between leaf resistance traits and measures of herbivory. Genetic correlations (r_g) were calculated from a multi-response model with the additive genetic relationship matrix as a random effect. Standard errors (SE) were calculated using the delta method. *P* was calculated by comparing the full model to a model in which r_g was fixed at zero using a likelihood ratio test.

Trait pair	r_g	SE	χ^2	*P*
Leaf ash, change in caterpillar mass	−0.289	0.305	1.323	0.250
Leaf ash, field leaf damage	0.110	0.254	0.261	0.610
LMA, change in caterpillar mass	−0.315	0.222	2.946	**0.086**
LMA, field leaf damage	−0.140	0.176	0.800	0.371
Leaf ash, LMA	0.215	0.265	0.693	0.405
Change in caterpillar mass, leaf mass consumed	0.464	0.499	1.132	0.287
Change in caterpillar mass, field leaf damage	0.472	0.194	7.981	**0.005**
Leaf mass consumed, field leaf damage	−0.019	0.490	<0.001	0.994

These results indicate that LMA was a stronger contributor to herbivore resistance than leaf ash. LMA was negatively genetically correlated with both change in caterpillar mass ($r_g = –0.315$) and field leaf damage ($r_g = –0.140$), indicating a potential role of LMA in resistance to herbivory. The relationship between leaf ash and measures of herbivory was mixed: leaf ash was negatively correlated with change in caterpillar mass ($r_g = –0.289$) and slightly positively correlated with field leaf damage ($r_g = 0.110$). Finally, LMA and leaf ash did not significantly genetically covary ($r_g = 0.215$), which is consistent with the fact that the two leaf resistance traits were genetically correlated with different measures of herbivory.

Are measures of herbivory positively genetically correlated with one another?

One possible explanation for the inconsistent results across different measures of herbivory was that they were not always positively related to one another genetically (Table 1). Change in caterpillar mass was positively correlated with field leaf damage ($r_g = 0.472$) and with leaf mass consumed ($r_g = 0.464$). Field damage, though, was uncorrelated with leaf mass consumed ($r_g = –0.019$).

Which genomic regions are associated with physical resistance traits and measures of herbivory?

Genome-wide association mapping with a 10% false-discovery rate threshold detected significant SNPs associated with all three measures of herbivory. Specifically, we found genomic regions associated with field damage across 8 of 18 chromosomes (Figure 5A; Appendix S2). There were 24 genes within 10 kb of a significant SNP, including a gene that functions in cation exchange (Pavir.3KG265695) and a gene that functions in pentatricopeptide expression (Pavir.9NG254200). At the more relaxed 15% false discovery rate threshold, there were also genes associated with ATP-citrate lyase (Pavir.5NG212200) and nitrate reductase 1 (Pavir.6KG411500). Five genomic regions were associated with leaf mass consumed (Figure 5B; Appendix S2), including a gene for TGACG motif-binding factor 6 (Pavir.9NG610300) and a gene for defensin-like peptides (Pavir.1KG388800). One SNP was associated with change in caterpillar mass (Figure 5C; Appendix S2). This SNP had two nearby candidate genes: AUX/IAA transcriptional regulator family protein (Pavir.5KG064800) and protein phosphatase 2A regulatory B subunit family protein (Pavir.5KG064900). Among the SNPs associated with different measures of herbivory, none colocalized on the same region of a chromosome, indicating a lack of evidence for any single molecular function determining multiple aspects of herbivory (i.e., no evidence for pleiotropic effects were detected). Unlike the three measures of herbivory, there were no SNPs significantly associated with LMA. Moreover, only one genomic region was associated with leaf ash (Pavir.4KG048905; BAH domain containing protein; Appendix S1, Figure S1; Appendix S2).

DISCUSSION

These results improve our understanding of the LHDH by highlighting differences in the way that latitudinal gradients in herbivory operate among and within subpopulations. When all genotypes were considered together, results were largely consistent with the LHDH—caterpillars gained more mass on and caused more damage in the field to higher-latitude *P. virgatum* genotypes (Moles et al., 2011a; Anstett et al., 2016; Baskett and Schemske, 2018). Subpopulation comparisons also supported the LHDH—the northernmost subpopulation (Midwest) generally experienced more herbivory than the southernmost subpopulation (Gulf). However, the LHDH was far less consistent within genetic subpopulations of *P. virgatum*. One reason that hypotheses like the LHDH can break down within species is that populations of a species often occupy distinct habitat types in different portions of their ranges rather than habitats that form a smooth environmental gradient (Agrawal, 2020). In *P. virgatum*, for instance, plants of the Gulf subpopulation typically occur in mesic, lowland habitats, while plants of the Midwest subpopulation typically occur in xeric, upland habitats (Lowry et al., 2014; Lovell et al., 2021). These subpopulations, which occupy different habitats across relatively narrow geographic ranges, give rise to morphologically distinct plants (Aspinwall et al., 2013; Lovell et al., 2021). It is not surprising, then, that significant within-subpopulation trends were less common than trends across the entire range of the species. In fact, the most

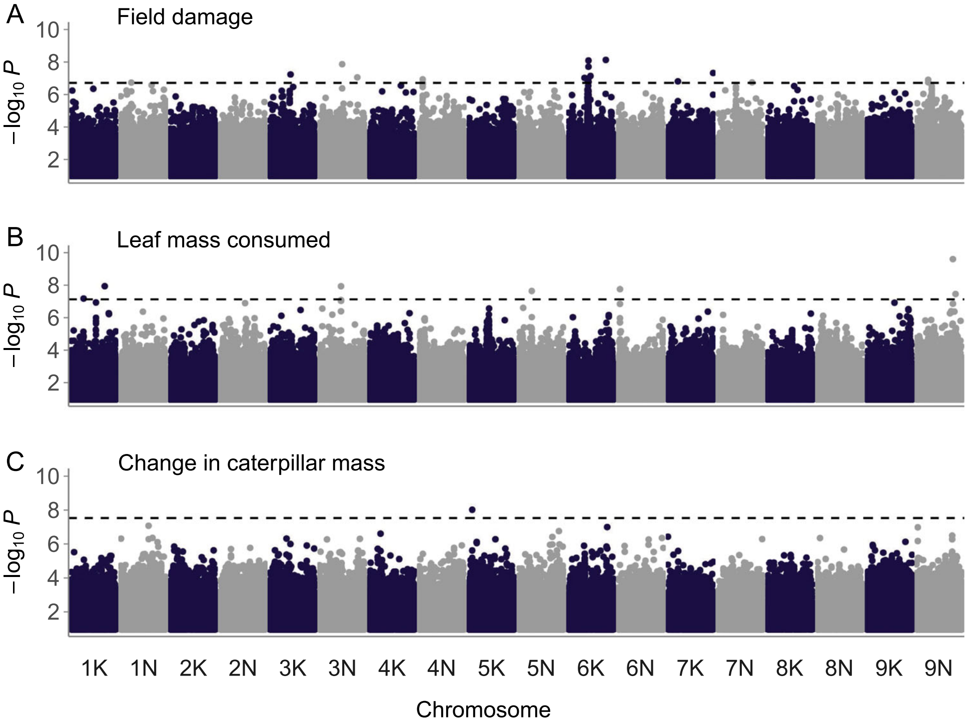

FIGURE 5 Manhattan plots showing the association between single nucleotide polymorphisms (SNPs) and (A) field leaf damage, (B) leaf mass consumed by caterpillars in the lab, and (C) change in caterpillar mass in the lab. Each point represents a SNP; location of each point along the *x*-axis represents its physical position on the chromosome. The dashed line represents a genome-wide 10% false discovery rate threshold; SNPs above this line have a significant statistical association with the phenotype ($-\log_{10} P$). "K" and "N" on chromosome labels denote the subgenome from which each chromosome of the allotetraploid *P. virgatum* originated. SNPs for which $-\log_{10} P < 1$ were not plotted.

consistent within-subpopulation evidence for the LHDH occurred in the Atlantic subpopulation—the subpopulation with the largest latitudinal extent and the most phenotypic variation.

Two leaf resistance traits, LMA and leaf ash, had impacts on herbivory, which often differed among subpopulations. Physical leaf defenses are often very important in grasses, a group in which chemical defenses are frequently less prevalent (McNaughton et al., 1985; Tscharntke and Greiler, 1995). Leaf mass per area is often positively correlated with mechanical properties that make leaves difficult for chewing insects like *S. frugiperda* to tear, including in *P. virgatum* (Schuh et al., 2018). Here, LMA was substantially higher in the Gulf subpopulation than in the other subpopulations; however, LMA was not significantly associated with herbivory in the Gulf subpopulation. Perhaps this lack of an association was driven by the generally low levels of herbivory seen on Gulf plants—Gulf leaves exhibited relatively little variation in LMA and may have been so difficult to consume that discernable trends were absent. The negative genetic correlation between LMA and change in caterpillar mass suggests that in addition to increasing leaf toughness, LMA may also impose a secondary barrier to herbivory—indigestibility—which prevents the herbivore from assimilating leaf tissue into body mass (Farmer, 2014). The importance of LMA as a deterrent to herbivory is clear, though, across subpopulations: high-LMA plants had considerably lower change in caterpillar mass and field damage at both the phenotypic and genetic levels.

Similarly, leaf ash, a proxy for silica content, is often an important determinant of resistance to herbivory (Moles et al., 2011b). Silica is a major contributor to leaf toughness and some chemical defenses (Nabity et al., 2011; Hall et al., 2019). Here, leaf ash was negatively associated with leaf mass consumed, but unlike LMA, it was not negatively associated with change in caterpillar mass. These results suggest that increased silica could present a physical barrier to chewing leaves, but not a barrier to caterpillars getting nourishment from eating the leaves. Thus, both traits may act in complementary ways to influence different aspects of resistance to herbivory (Agrawal and Fishbein, 2006). Overall, the impact of leaf ash on herbivory was inconsistent, especially within subpopulations. These results may have been partly related to the inducibility of leaf silica. If field herbivory induced additional production of chemical or physical defenses (Massey et al., 2007), it could have impacted the leaves used in the subsequent lab feeding trials. Induced silica production could potentially explain the unexpected significant phenotypic relationship between leaf ash (measured in 2019) and damage in the field (measured in 2018), which was contrary to other studies in *P. virgatum* (e.g., Nabity et al., 2011). In our study, the Midwest plants had the highest leaf ash in 2019 and the highest field damage in 2018. Thus, it is possible that the high heritability of leaf ash in this study resulted partly from the induction of silica uptake in the Midwest plants that had experienced heavy herbivory the previous year (Reynolds et al., 2012). Given the extremely high heritability associated with field damage ($h^2 = 0.840$), an entirely inducible response to herbivory, primarily in the Midwest subpopulation, could have been observed as highly heritable. Follow-up studies could examine genetic correlations in leaf ash between individuals that varied in exposure to herbivory.

Our inconsistent support for the LHDH can be partly attributed to differences in the ways that we quantified herbivory. For instance, lab-based feeding trials can be rigorously controlled (e.g., duration, environmental conditions, herbivore mass, or developmental stage), but often lack realism, especially in no-choice assays (Boyd, 2007). On the other hand, field observations represent natural conditions, but can lack experimental rigor (Bergelson et al., 2021; Howard et al., 2022). In field studies, extraneous factors that can impact herbivory, like environmental stress, previous pathogen infection, or location within a field, can be difficult to control. One result that highlights the importance of examining multiple aspects of herbivory was the amount of Gulf subpopulation leaf mass that caterpillars consumed in the lab. In most other measures, the Gulf subpopulation had extreme values relative to the other genotypes, yet caterpillars consumed similar amounts of leaf mass across subpopulations. This suggests that herbivores may consume more tissue from a low-quality leaf and still not perform as well as they would when consuming less tissue from a highly nutritious leaf (Mattson, 1980; Berner et al., 2005; La Pierre and Smith, 2016). If given a choice, it is likely that the caterpillars—or ovipositing female moths in the field—would choose more nutritious genotypes (Jaenike, 1978; Gripenberg et al., 2010; but see Rojas et al., 2018). This hypothesis is consistent with our field damage results: one candidate gene associated with field damage (at the 15% false discovery rate threshold) is related to nitrate reductase 1 (Pavir.6KG411500), which can increase leaf protein (Davenport et al., 2015).

The decision of which leaves to consume may have population- and community-level consequences (Crawley, 1989; Fine et al., 2004; Koerner et al., 2018). Plant genotypes that are more nutritious will also increase the rate at which caterpillars develop and increase their size at pupation, both of which should increase the growth rate of the herbivore population (White, 1974; Coley et al., 2006). Larger herbivore populations may then impose stronger top-down regulation on plant populations (Hairston et al., 1960; Crawley, 1989), which suggests that caterpillar performance was the most ecologically relevant measure of herbivory in our lab-based feeding trial. Moreover, change in caterpillar mass and field damage were positively genetically correlated and both provided evidence that the Gulf genetic subpopulation was more resistant to herbivory than either the Midwest or the Atlantic subpopulation. Taken together, the combination of field and lab observations can provide valuable insight and a fuller picture of the relationship between plants and herbivores (Pérez-Harguindeguy et al., 2003).

Impact of herbivory defense on bioenergy utility

In addition to being an important component of North American grasslands, *P. virgatum* is also a candidate bioenergy crop (Casler, 2012). In a bioenergy crop, high resistance to herbivory is often desirable, but when traits that confer resistance to herbivory also reduce the efficiency with which biomass is converted into ethanol, they could limit biofuel production (Jakob et al., 2009; Buanafina and Fescemyer, 2012). For instance, high leaf ash tends to reduce the efficiency of biomass conversion to biofuels (Monti et al., 2008; Lacey et al., 2016). On the other hand, evidence suggests that leaf toughness does not impact enzymatic degradation for biofuel production (Zhang et al., 2015; Głazowska et al., 2018). Thus, selecting for low-ash genotypes that exhibit high resistance to herbivory through another mechanism would be a promising approach. Several candidate genes that were associated with different measures of herbivory contribute to molecular mechanisms of plant defense. For instance, pentatricopeptide expression (Pavir.9NG254200) is involved in the jasmonic acid defense pathway in rice (Guo et al., 2015), while protein phosphatase 2A regulatory subunit B (Pavir.5KG064900) impacts susceptibility to aphid herbivory in *Arabidopsis thaliana* (Rasool et al., 2014). Because these pathways are not directly related to physical resistance, they may reduce herbivory without also reducing conversion efficiency.

CONCLUSIONS

This study contributes to the findings of several recent studies that have identified discrepancies in the LHDH within species, showing that broad hypotheses about defense investment may be less applicable at this level (Anstett et al., 2014; Kooyers et al., 2017). Specifically, here we found that latitudinal gradients in defensive strategies may operate differently among and within subpopulations of *P. virgatum*. This lack of consistency with the LHDH within genetic subpopulations may result from our choice of resistance traits—leaf ash and LMA contributed to different aspects of resistance to herbivory in *P. virgatum*—as well as the different ecological conditions and evolutionary histories of the three genetic subpopulations of *P. virgatum*. An important goal moving forward, then, should be to identify the conditions under which the LHDH and similar hypotheses are valid within species or closely related higher taxa to better understand the factors driving the evolution of defensive strategies.

AUTHOR CONTRIBUTIONS

T.E.J. and R.W.H. conceived the study; K.C.H. and R.W.H. implemented the experiment and analyzed the data; K.C.H. and R.W.H. wrote the manuscript with contributions from T.E.J.

ACKNOWLEDGMENTS

We thank Matthew Carey, David Rowley, Jason Bonnette, Anne Gibson, and Philip Fay for field and logistical support. We also thank Barbara Demmig-Adams, Cam Durant, Pieter Johnson, Jennifer Knight, and three anonymous reviewers for helpful comments on previous drafts. This research was supported by the Office of Science (BER), U.S. Department of Energy, grant no. DE-SC0014156; by the National Science Foundation Plant Genome Research Program, grant no. IOS-1444533; and in part by the U.S. Department of Agriculture Forest Service, Rocky Mountain Research Station. The findings and conclusions of this publication are those of the authors and should not be construed to represent any official USDA or U.S. Government determination or policy.

DATA AVAILABILITY STATEMENT

Data associated with this study are available from Figshare: https://doi.org/10.6084/m9.figshare.24424618.

ORCID

Kevin C. Headrick http://orcid.org/0000-0002-8042-1168
Thomas E. Juenger http://orcid.org/0000-0001-9550-9288
Robert W. Heckman http://orcid.org/0000-0002-2281-3091

REFERENCES

Agrawal, A. A. 2020. A scale-dependent framework for trade-offs, syndromes, and specialization in organismal biology. *Ecology* 101: e02924.

Agrawal, A. A., and M. Fishbein. 2006. Plant defense syndromes. *Ecology* 87: S132-S149.

Anderson, J. T., and T. Mitchell-Olds. 2011. Ecological genetics and genomics of plant defences: evidence and approaches. *Functional Ecology* 25:312-324.

Anderson, J. T., J. H. Willis, and T. Mitchell-Olds. 2011. Evolutionary genetics of plant adaptation. *Trends in Genetics* 27: 258-266.

Anstett, D. N., J. R. Ahern, J. Glinos, N. Nawar, J.-P. Salminen, and M. T. J. Johnson. 2015. Can genetically based clines in plant defence explain greater herbivory at higher latitudes? *Ecology Letters* 18: 1376-1386.

Anstett, D. N., I. Naujokaitis-Lewis, and M. T. J. Johnson. 2014. Latitudinal gradients in herbivory on *Oenothera biennis* vary according to herbivore guild and specialization. *Ecology* 95: 2915-2923.

Anstett, D. N., K. A. Nunes, C. Baskett, and P. M. Kotanen. 2016. Sources of controversy surrounding latitudinal patterns in herbivory and defense. *Trends in Ecology & Evolution* 31: 789-802.

Aspinwall, M. J., D. B. Lowry, S. H. Taylor, T. E. Juenger, C. V. Hawkes, M.-V. V. Johnson, J. R. Kiniry, and P. A. Fay. 2013. Genotypic variation in traits linked to climate and aboveground productivity in a widespread C_4 grass: evidence for a functional trait syndrome. *New Phytologist* 199: 966-980.

Baskett, C. A., and D. W. Schemske. 2018. Latitudinal patterns of herbivore pressure in a temperate herb support the biotic interactions hypothesis. *Ecology Letters* 21: 578-587.

Bergelson, J., M. Kreitman, D. A. Petrov, A. Sanchez, and M. Tikhonov. 2021. Functional biology in its natural context: a search for emergent simplicity. *eLife* 10: e67646.

Berner, D., W. U. Blanckenhorn, and C. Körner. 2005. Grasshoppers cope with low host plant quality by compensatory feeding and food selection: N limitation challenged. *Oikos* 111: 525-533.

Boyd, R. S. 2007. The defense hypothesis of elemental hyperaccumulation: status, challenges and new directions. *Plant and Soil* 293: 153-176.

Buanafina, M. M. d. O., and H. W. Fescemyer. 2012. Modification of esterified cell wall phenolics increases vulnerability of tall fescue to herbivory by the fall armyworm. *Planta* 236: 513-523.

Capinera, J. L. 2002. Fall armyworm, *Spodoptera frugiperda* (JE Smith) (Insecta: Lepidoptera: Noctuidae): EENY098/IN255, rev. 7/2000. EDIS. Website: https://edis.ifas.ufl.edu/publication/IN255

Casler, M. D. 2012. Switchgrass breeding, genetics, and genomics. *In* A. Monti [ed.], Switchgrass: A valuable biomass crop for energy, 29–53. Springer London, UK.

Coley, P. D., and J. A. Barone. 1996. Herbivory and plant defenses in tropical forests. *Annual Review of Ecology and Systematics* 27: 305-335.

Coley, P. D., M. L. Bateman, and T. A. Kursar. 2006. The effects of plant quality on caterpillar growth and defense against natural enemies. *Oikos* 115: 219-228.

Covarrubias-Pazaran, G. 2016. Genome-assisted prediction of quantitative traits using the R package sommer. *PLoS One* 11: e0156744.

Crawley, M. J. 1989. Insect herbivores and plant population dynamics. *Annual Review of Entomology* 34: 531-562.

Croy, J. R., J. D. Pratt, and K. A. Mooney. 2022. Latitudinal resource gradient shapes multivariate defense strategies in a long-lived shrub. *Ecology* 103: e3830.

Davenport, S., P. Le Lay, and J. P. Sanchez-Tamburrrino. 2015. Nitrate metabolism in tobacco leaves overexpressing Arabidopsis nitrite reductase. *Plant Physiology and Biochemistry* 97: 96-107.

Deshmukh, S. S., B. M. Prasanna, C. M. Kalleshwaraswamy, J. Jaba, and B. Choudhary. 2021. Fall armyworm (*Spodoptera frugiperda*). *In* Omkar [ed.], Polyphagous pests of crops, 349–372. Springer, Singapore.

Dowd, P. F., and E. T. Johnson. 2009. Differential resistance of switchgrass *Panicum virgatum* L. lines to fall armyworms *Spodoptera frugiperda*. *Genetic Resources and Crop Evolution* 56: 1077-1089.

Dowd, P. F., G. Sarath, R. B. Mitchell, A. J. Saathoff, and K. P. Vogel. 2013. Insect resistance of a full sib family of tetraploid switchgrass *Panicum virgatum* L. with varying lignin levels. *Genetic Resources and Crop Evolution* 60: 975-984.

Erb, M., C. A. M. Robert, B. E. Hibbard, and T. C. J. Turlings. 2011. Sequence of arrival determines plant-mediated interactions between herbivores. *Journal of Ecology* 99: 7-15.

Farmer, E. E. 2014. Leaf defence. Oxford University Press, Oxford, UK.

Fine, P. V. A., I. Mesones, and P. D. Coley. 2004. Herbivores promote habitat specialization by trees in Amazonian forests. *Science* 305: 663-665.

Głazowska, S., L. Baldwin, J. Mravec, C. Bukh, T. H. Hansen, M. M. Jensen, J. U. Fangel, et al. 2018. The impact of silicon on cell wall composition and enzymatic saccharification of Brachypodium distachyon. *Biotechnology for Biofuels* 11: 171.

Gripenberg, S., P. J. Mayhew, M. Parnell, and T. Roslin. 2010. A meta-analysis of preference–performance relationships in phytophagous insects. *Ecology Letters* 13: 383-393.

Guo, H.-M., S.-C. Sun, F.-M. Zhang, and X.-X. Miao. 2015. Identification of genes potentially related to herbivore resistance in OPR3 overexpression rice by microarray analysis. *Physiological and Molecular Plant Pathology* 92: 166-174.

Hairston, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control, and competition. *American Naturalist* 94: 421-425.

Hall, C. R., J. M. Waterman, R. K. Vandegeer, S. E. Hartley, and S. N. Johnson. 2019. The role of silicon in antiherbivore phytohormonal signalling. *Frontiers in Plant Science* 10: 1132.

Hanley, M. E., B. B. Lamont, M. M. Fairbanks, and C. M. Rafferty. 2007. Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics* 8: 157-178.

Hartley, S. E., and J. L. DeGabriel. 2016. The ecology of herbivore-induced silicon defences in grasses. *Functional Ecology* 30: 1311-1322.

He, L.-M., T.-L. Wang, Y.-C. Chen, S.-S. Ge, K. A. G. Wyckhuys, and K.-M. Wu. 2021. Larval diet affects development and reproduction of East Asian strain of the fall armyworm, *Spodoptera frugiperda*. *Journal of Integrative Agriculture* 20: 736-744.

Heckman, R. W., F. W. Halliday, and C. E. Mitchell. 2019. A growth–defense trade-off is general across native and exotic grasses. *Oecologia* 191: 609-620.

Heckman, R. W., A. R. Khasanova, N. S. Johnson, S. Weber, J. E. Bonnette, M. J. Aspinwall, L. G. Reichmann, et al. 2020. Plant biomass, not plant economics traits, determines responses of soil CO_2 efflux to precipitation in the C_4 grass *Panicum virgatum*. *Journal of Ecology* 108: 2095-2106.

Howard, M. M., E. Bass, A. Chautá, D. Mutyambai, and A. Kessler. 2022. Integrating plant-to-plant communication and rhizosphere microbial dynamics: ecological and evolutionary implications and a call for experimental rigor. *ISME Journal* 16: 5-9.

Jaenike, J. 1978. On optimal oviposition behavior in phytophagous insects. *Theoretical Population Biology* 14: 350-356.

Jakob, K., F. Zhou, and A. H. Paterson. 2009. Genetic improvement of C_4 grasses as cellulosic biofuel feedstocks. *In Vitro Cellular & Developmental Biology - Plant* 45: 291-305.

Kikuzawa, K., Y. Onoda, I. J. Wright, and P. B. Reich. 2013. Mechanisms underlying global temperature-related patterns in leaf longevity. *Global Ecology and Biogeography* 22: 982-993.

Koerner, S. E., M. D. Smith, D. E. Burkepile, N. P. Hanan, M. L. Avolio, S. L. Collins, A. K. Knapp, et al. 2018. Change in dominance determines herbivore effects on plant biodiversity. *Nature Ecology & Evolution* 2: 1925-1932.

Kooyers, N. J., B. K. Blackman, and L. M. Holeski. 2017. Optimal defense theory explains deviations from latitudinal herbivory defense hypothesis. *Ecology* 98: 1036-1048.

La Pierre, K. J., and M. D. Smith. 2016. Soil nutrient additions increase invertebrate herbivore abundances, but not herbivory, across three grassland systems. *Oecologia* 180: 485-497.

Lacey, J. A., R. M. Emerson, D. N. Thompson, and T. L. Westover. 2016. Ash reduction strategies in corn stover facilitated by anatomical and size fractionation. *Biomass and Bioenergy* 90: 173-180.

Lanning, F. C., and L. N. Eleuterius. 1983. Silica and ash in tissues of some coastal plants. *Annals of Botany* 51: 835-850.

Lanning, F. C., and L. N. Eleuterius. 1987. Silica and ash in native plants of the central and southeastern regions of the United States. *Annals of Botany* 60: 361-375.

Lenth, R. 2022. emmeans: Estimated marginal means, aka least-squares means. Website: https://CRAN.R-project.org/package=emmeans

Li, H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* 25: 1754-1760.

Lovell, J. T., A. H. MacQueen, S. Mamidi, J. Bonnette, J. Jenkins, J. D. Napier, Avinash Sreedasyam, et al. 2021. Genomic mechanisms of climate adaptation in polyploid bioenergy switchgrass. *Nature* 590: 438-444.

Lowry, D. B., K. D. Behrman, P. Grabowski, G. P. Morris, J. R. Kiniry, and T. E. Juenger. 2014. Adaptations between ecotypes and along environmental gradients in *Panicum virgatum*. *American Naturalist* 183: 682-692.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA, USA.

Mackay, T. F. C. 2001. The genetic architecture of quantitative traits. *Annual Review of Genetics* 35: 303-339.

Massey, F. P., A. Roland Ennos, and S. E. Hartley. 2007. Herbivore specific induction of silica-based plant defences. *Oecologia* 152: 677-683.

Mattson, W. J. 1980. Herbivory in relation to plant-nitrogen content. *Annual Review of Ecology and Systematics* 11: 119-161.

Mauricio, R. 2001. Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. *Nature Reviews Genetics* 2: 370-381.

McNaughton, S. J., J. L. Tarrants, M. M. McNaughton, and R. D. Davis. 1985. Silica as a defense against herbivory and a growth promotor in African grasses. *Ecology* 66: 528-535.

Mithöfer, A., and W. Boland. 2012. Plant defense against herbivores: Chemical aspects. *Annual Review of Plant Biology* 63: 431-450.

Moles, A. T., S. P. Bonser, A. G. B. Poore, I. R. Wallis, and W. J. Foley. 2011a. Assessing the evidence for latitudinal gradients in plant defence and herbivory. *Functional Ecology* 25: 380-388.

Moles, A. T., B. Peco, I. R. Wallis, W. J. Foley, A. G. B. Poore, E. W. Seabloom, P. A. Vesk, et al. 2013. Correlations between physical and chemical defences in plants: tradeoffs, syndromes, or just many different ways to skin a herbivorous cat? *New Phytologist* 198: 252-263.

Moles, A. T., I. R. Wallis, W. J. Foley, D. I. Warton, J. C. Stegen, A. J. Bisigato, L. Cella-Pizarro, et al. 2011b. Putting plant resistance traits on the map: a test of the idea that plants are better defended at lower latitudes. *New Phytologist* 191: 777-788.

Monti, A., N. Di Virgilio, and G. Venturi. 2008. Mineral composition and ash content of six major energy crops. *Biomass and Bioenergy* 32: 216-223.

Moreno, J. E., Y. Tao, J. Chory, and C. L. Ballaré. 2009. Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. *Proceedings of the National Academy of Sciences, USA* 106: 4935-4940.

Nabity, P. D., R. Orpet, S. Miresmailli, M. R. Berenbaum, and E. H. DeLucia. 2012. Silica and nitrogen modulate physical defense against chewing insect herbivores in bioenergy crops *Miscanthus* × *giganteus* and *Panicum virgatum* (Poaceae). *Journal of Economic Entomology* 105: 878-883.

Nabity, P. D., A. R. Zangerl, M. R. Berenbaum, and E. H. DeLucia. 2011. Bioenergy crops *Miscanthus* × *giganteus* and *Panicum virgatum* reduce growth and survivorship of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 104: 459-464.

Napier, J. D., R. W. Heckman, and T. E. Juenger. 2023. Gene-by-environment interactions in plants: molecular mechanisms, environmental drivers, and adaptive plasticity. *Plant Cell* 35: 109–124.

Onoda, Y., M. Westoby, P. B. Adler, A. M. F. Choong, F. J. Clissold, J. H. C. Cornelissen, S. Díaz, et al. 2011. Global patterns of leaf mechanical properties. *Ecology Letters* 14: 301-312.

Parker, J. D., J. P. Salminen, and A. A. Agrawal. 2012. Evolutionary potential of root chemical defense: genetic correlations with shoot chemistry and plant growth. *Journal of Chemical Ecology* 38: 992-995.

Pérez-Harguindeguy, N., S. Díaz, E. Garnier, S. Lavorel, H. Poorter, P. Jaureguiberry, M. S. Bret-Harte, et al. 2013. New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany* 61: 167-234.

Pérez-Harguindeguy, N., S. Díaz, F. Vendramini, J. H. C. Cornelissen, D. E. Gurvich, and M. Cabido. 2003. Leaf traits and herbivore selection in the field and in cafeteria experiments. *Austral Ecology* 28: 642-650.

R Core Team. 2023. R: A language and environment for statistical computing.R Foundation for Statistical Computing.

Rasmann, S., and A. A. Agrawal. 2011. Latitudinal patterns in plant defense: evolution of cardenolides, their toxicity and induction following herbivory. *Ecology Letters* 14: 476-483.

Rasool, B., B. Karpinska, G. Konert, G. Durian, K. Denessiouk, S. Kangasjärvi, and C. H. Foyer. 2014. Effects of light and the regulatory B-subunit composition of protein phosphatase 2A on the susceptibility of *Arabidopsis thaliana* to aphid (*Myzus persicae*) infestation. *Frontiers in Plant Science* 5: 405.

Reynolds, J. J. H., X. Lambin, F. P. Massey, S. Reidinger, J. A. Sherratt, M. J. Smith, A. White, and S. E. Hartley. 2012. Delayed induced silica defences in grasses and their potential for destabilising herbivore population dynamics. *Oecologia* 170: 445-456.

Rojas, J. C., M. V. Kolomiets, and J. S. Bernal. 2018. Nonsensical choices? Fall armyworm moths choose seemingly best or worst hosts for their larvae, but neonate larvae make their own choices. *PLoS One* 13: e0197628.

Schemske, D. W., G. G. Mittelbach, H. V. Cornell, J. M. Sobel, and K. Roy. 2009. Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution and Systematics* 40: 245-269.

Schlum, K. A., K. Lamour, C. P. de Bortoli, R. Banerjee, R. Meagher, E. Pereira, M. G. Murua, et al. 2021. Whole genome comparisons reveal panmixia among fall armyworm (*Spodoptera frugiperda*) from diverse locations. *BMC Genomics* 22: 179.

Schuh, M. K., C. A. Bahlai, C. M. Malmstrom, and D. A. Landis. 2018. Effect of switchgrass ecotype and cultivar on establishment, feeding, and development of fall armyworm (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 112: 440-449.

Storey, J. D., and R. Tibshirani. 2003. Statistical significance for genome-wide studies. *Proceedings of the National Academy of Sciences, USA* 100: 9440-9445.

Sun, X.-X., C.-X. Hu, H.-R. Jia, Q.-L. Wu, X.-J. Shen, S.-Y. Zhao, Y. Y. Jiang, and K.-M. Wu. 2021. Case study on the first immigration of fall armyworm, *Spodoptera frugiperda*, invading into China. *Journal of Integrative Agriculture* 20: 664-672.

Tibbs Cortes, L., Z. Zhang, and J. Yu. 2021. Status and prospects of genome-wide association studies in plants. *Plant Genome* 14: e20077.

Travers-Martin, N., and C. Müller. 2008. Matching plant defence syndromes with performance and preference of a specialist herbivore. *Functional Ecology* 22: 1033-1043.

Tscharntke, T., and H. J. Greiler. 1995. Insect communities, grasses, and grasslands. *Annual Review of Entomology* 40: 535-558.

VanRaden, P. M. 2008. Efficient methods to compute genomic predictions. *Journal of Dairy Science* 91: 4414-4423.

VanWallendael, A., J. Bonnette, T. E. Juenger, F. B. Fritschi, P. A. Fay, R. B. Mitchell, J. Lloyd-Reilley, et al. 2020. Geographic variation in the genetic basis of resistance to leaf rust between locally adapted ecotypes of the biofuel crop switchgrass (*Panicum virgatum*). *New Phytologist* 227: 1696-1708.

Walsh, B., and M. W. Blows. 2009. Abundant genetic variation + strong selection = multivariate genetic constraints: a geometric view of adaptation. *Annual Review of Ecology, Evolution, and Systematics* 40: 41-59.

White, T. C. R. 1974. A hypothesis to explain outbreaks of looper caterpillars, with special reference to populations of *Selidosema suavis* in a plantation of *Pinus radiata* in New Zealand. *Oecologia* 16: 279-301.

Wilson, A. J., D. Réale, M. N. Clements, M. M. Morrissey, E. Postma, C. A. Walling, L. E. B. Kruuk, and D. H. Nussey. 2010. An ecologist's guide to the animal model. *Journal of Animal Ecology* 79: 13-26.

Zhang, J., W. Zou, Y. Li, Y. Feng, H. Zhang, Z. Wu, Y. Tu, et al. 2015. Silica distinctively affects cell wall features and lignocellulosic saccharification with large enhancement on biomass production in rice. *Plant Science* 239: 84-91.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Statistical tables and figures.

Appendix S2. List of single nucleotide polymorphisms (SNPs) within 10-kb pairs of a significant SNP in genome-wide association mapping.

How to cite this article: Headrick, K. C., T. E. Juenger, and R. W. Heckman. 2024. Plant physical defenses contribute to a latitudinal gradient in resistance to insect herbivory within a widespread perennial grass. *American Journal of Botany* 111(1): e16260. https://doi.org/10.1002/ajb2.16260