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Research article

Evolutionary trajectories of multiple defense traits across phylogenetic and geographic scales in *Vitis*

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The processes driving defense trait correlations may vary within and between species based on ecological or environmental contexts. However, most studies of plant defense theory fail to address this potential for shifts in trait correlations across scales. In this work, we tested for correlations between multiple defensive traits (secondary chemistry, carbon to nitrogen ratio, domatia, leaf toughness, trichomes, and pearl bodies) across a common garden of 21 *Vitis* species and eighteen genotypes of the species *Vitis riparia* to identify when and where patterns of defense trait evolution persist or break down across biological scales. Additionally, we asked whether *Vitis* defense trait investment correlates with environmental variables as predicted by plant defense theory, using environmental metrics for each *Vitis* species and *V. riparia* genotype from the GBIF and WorldClim databases. We tested for correlations between defense trait investment, herbivore palatability, and environmental variables using phylogenetically informed models. Beyond a few likely physiological exceptions, we observed a lack of significant correlations between defense traits at both intra- and interspecific scales, indicating that these traits evolve independently of each other in *Vitis* rather than forming predictable defense syndromes. We did find that investment in carbon:nitrogen (at both scales) and pearl bodies increases with proximity to the equator, demonstrating support for plant defense theory's prediction of higher investment in defenses at more equatorial environments for some, but not all, defense traits. Overall, our results challenge commonly held hypotheses about plant defense evolution, namely the concept of syndromes, by demonstrating that strong correlations between defense traits are not the prevailing pattern both across and within *Vitis* species. Our work also provides the first comprehensive evaluation of the evolutionary divergence in approaches that *Vitis*, a genus with significant agricultural value, have evolved to defend themselves against herbivores.

Keywords: chemical defense, direct defense, domatia, herbivory, indirect defense, pearl bodies, phylogenetic comparative methods, *Vitis*

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Introduction

Plants display an impressive array of traits to reduce or deter herbivory, producing sophisticated defense arsenals that make up for their lack of mobility to escape damage. There are many hypothesized drivers of this stunning diversity of defense traits, but plant defense theory generally neglects to predict what drivers of diversity dominate at different levels of biological scale (i.e. within versus across plant species). Recent theory has proposed that the processes that shape trait correlations are likely to shift across environmental, spatial, and temporal sample scales, thereby influencing what patterns we detect and ultimately the conclusions we come to about the drivers of trait divergence when we look only within *or* across species (Laughlin et al. 2017, Agrawal 2020, Zhou et al. 2022). For example, a given resource may be limiting at the within species scale, manifesting as a negative correlation between traits, while that same resource may be more available at the between species scale, thereby attenuating the negative trait correlation (Hahn and Maron 2016, Zhou et al. 2022). Thus, we may see different evolutionary correlations between the same defense traits depending on whether we are comparing individuals within a species versus between species.

Theory about the drivers of defense variation often focuses on aspects of the plants' biology that are predicted to impact selection on defenses. For example, plants are hypothesized to specialize in certain defense strategies or traits at the expense of others, either as the result of resource-based tradeoffs, which prevent the simultaneous expression of two traits with the same metabolic precursor(s) (Agrawal et al. 2010, Agrawal 2020) or due to an ecological conflict between the traits (Strauss et al. 2002). For example, it might be disadvantageous to provide extrafloral nectar to mutualist arthropods if the toxic metabolites within a plant leak into that nectar and potentially poison the defenders. There are also many reasons to expect there to be positive correlations between defenses, oftentimes termed 'syndromes' (Kursar and Coley 2003, Agrawal and Fishbein 2006). Syndromes might occur when traits interact synergistically to provide defense (Berenbaum and Neal 1985, Rasmann and Agrawal 2009), are genetically or developmentally linked, or in situations where plants are subject to damage from a range of generalist and specialist herbivores (e.g. in the tropics, Schemske et al. 2009) resulting in multiple seemingly-redundant defense traits being advantageous.

Geographic patterns in environmental variables are also hypothesized drivers of defense trait evolution, although these patterns are also rarely examined across scales. For example, one prominent theory, sometimes referred to as the latitudinal herbivory defense hypothesis (LHDH), predicts that plants invest more in defenses closer to the equator because the tropics are diversity hotspots with high levels of herbivory pressure (Coley and Aide 1991, Coley and Barone 1996, Anstett et al. 2016). Equatorial climes also have longer growing seasons, lengthening the amount of time that plants are threatened by arthropod herbivores. Resource availability may also influence the evolution of defense traits in plants. Plants are often observed to be highly defended against herbivory in resource-poor environments, a pattern hypothesized to result from selection for plants to protect hard-won resources in their relatively expensive-to-produce photosynthetic tissue (the resource availability hypothesis, RAH; Coley et al. 1985, Endara and Coley 2011). While mean environmental temperature and growing season length typically vary closely with latitude, resource availability may not. Thus, the LHDH and the RAH may produce conflicting patterns of defense investment, such as in arid environments far from the equator where the growing seasons are short (suggesting that defense investment should be low) but nutrients are limited (suggesting that defense investment should be high; Coley et al. 1985).

Although rare, several studies have tested whether the theorized correlations between traits, or between traits and environmental variables, persist or break-down across levels of biological organization. In a 2012 meta-analysis of papers measuring correlations between direct and indirect defenses in plants that participate in defensive mutualisms using ants, Koricheva and Romero (2012) observed a negative correlation between the defense strategies that persisted both within and across species. In contrast, broad support has been found for the aforementioned resource availability at the interspecific level, but less so within species (Hahn and Maron 2016). Finally, Agrawal and Hastings (2019) found that a tradeoff between constitutive and induced chemical defenses is apparent within individual *Asclepias* species, but breaks down when sampling across species. These observations emphasize that the scale at which we choose to evaluate a pattern can give us different ideas of what factors shape the evolution of defense traits. Studies that address the scale-dependency of trait-trait and trait–environment correlations require thorough phenotyping of multiple traits across many species and individuals to comprehensively quantify defensive phenotypes in a plant group. As a result, few tests of plant defense theory test scale as an influence on the drivers that maintain or break-down associations between traits.

In this study, we aim to evaluate trait–trait and trait–environment correlations between an array of defense traits and environmental variables at both the inter- and intraspecific scale to explicitly test the scale-dependency of plant-defense syndromes. We do this using wild grape genus *Vitis* as our study system. *Vitis* is a charismatic, agriculturally important clade of about sixty species of woody vines, native to North America, Europe and Asia (Moore 1991, Zhiduan et al. 2007, Aradhya et al. 2013, Gerrath et al. 2015, Péros et al. 2023). *Vitis* vines have a wide range of traits thought to be directly and indirectly defensive against herbivores, making them an excellent system in which to investigate correlated defense trait evolution. We specifically ask at both the interspecific and intraspecific level 1) whether traits are correlated (positively or negatively) with one another, and 2) whether features of the abiotic environment predict defense trait investment. We do this by synthesizing defense trait quantifications, species occurrence data, climate data, and herbivore bioassays to achieve a holistic picture of defense in *Vitis* species, and ultimately gain insight into the evolutionary forces that are driving the evolution of *Vitis* defense traits at different levels of biological organization.

Material and methods

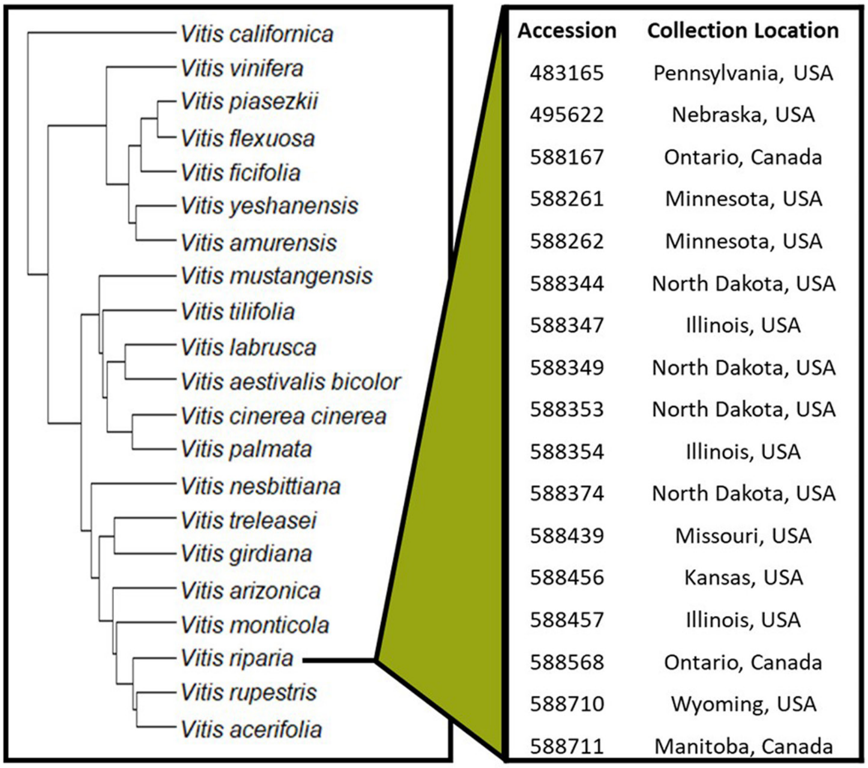
Experimental plants

We collected defense trait data on grapevines grown in a greenhouse common-garden. A common garden holds the environment constant, meaning that phenotypic variation between plants grown in that common environment can be assumed to be produced by genotypic variation between the plants. We attempted to growall *Vitis* species available from the USDA-ARS germplasm network, which we rooted as cuttings in the spring of 2021 (across genus vines) and 2022 (*V. riparia* vines). Two of the species in our data (*V. vinifera* and *V. rupestris*) are noted in the germplasm database as having been donated as cultivated material, while all others are either specified as collected as wild material or do not have this information available. The breadth of species of *Vitis* used in this study represent those we were able to obtain and root successfully (approximately one third of the ~ 60 species in the *Vitis* genus), and are thus biased towards species native to North America. However, the species in our dataset are representative of much of the latitudinal range of *Vitis*, and are native to the hotspots of *Vitis* diversity in North America and East Asia (Moore 1991, Zhiduan et al. 2007). We quantified defense metrics across 21 species of *Vitis* (1–6 individuals per species, mean = 4.38; all of the same genotype) and 18 *V. riparia* genotypes (4–5 individuals per *V. riparia* genotype, mean = 4.94; Fig. 1). All traits were collected on mature leaves, and, unless otherwise noted, we collected the trait data on a consistent area on the underside of each leaf adjacent to the midvein.

Metrics of plant defense

Chemistry

Numerous studies have demonstrated the negative impacts of specialized metabolites on herbivores by poisoning them, slowing their growth, or encouraging them to eat elsewhere (Levin 1976, Mithöfer and Boland 2012). We quantified grape secondary metabolism using liquid chromatography mass spectrometry (LCMS) of leaf extracts. We collected leaf punches from five mature leaves per experimental plant and pooled them so that we had one sample per plant that would capture a broader picture of the plant's metabolism. We immediately submerged the leaf punches in an extraction solution made up of isopropanol:acetonitrile:water 3:3:2 containing 0.1% formic acid and 104 nM telmisartan as an internal standard. We soaked the leaf material overnight, and then separated the extracts from the leaf tissue and stored the extracts at −20°C until analysis. We dried the remaining leaf tissue and measured its dry mass so we could standardize the quantity of metabolites per mass of leaf tissue.



Accession	Collection Location
483165	Pennsylvania, USA
495622	Nebraska, USA
588167	Ontario, Canada
588261	Minnesota, USA
588262	Minnesota, USA
588344	North Dakota, USA
588347	Illinois, USA
588349	North Dakota, USA
588353	North Dakota, USA
588354	Illinois, USA
588374	North Dakota, USA
588439	Missouri, USA
588456	Kansas, USA
588457	Illinois, USA
588568	Ontario, Canada
588710	Wyoming, USA
588711	Manitoba, Canada

Figure 1. *Vitis* species (left) and *V. riparia* genotypes (right) that we sampled to characterize multi-trait defense arsenals.

We analyzed the extracts (diluted 1:1 in ultrapure water) at the Michigan State University Mass Spectrometry and Metabolomics Core Facility using the facility's Thermo Q-Exactive mass spectrometer and Thermo Vanquish Flex UHPLC system with a Waters BEH-C18 reverse phase column. We used the same instrumentation and analysis parameters as reported in (Graham et al. 2023). The result of this analysis was raw peak data for each analyzed sample. We standardized all peak areas by subtracting out the peak areas of the averaged blanks, normalizing to the telmisartan peak, and dividing by the mass of the leaf tissue extracted. Additionally, we used the relative mass defects of the detected compounds to remove compounds with defects of over 1200, which are typically inorganic ions not of biological origin (Ekanayaka et al. 2015, Graham et al. 2023). Finally, we summarized the metabolic profiles of the samples in two ways: metabolite abundance (sum of the peak areas of all compounds in the sample), and metabolite richness (count of unique peaks in the samples).

Carbon-to-nitrogen ratio

We use C:N as a proxy for leaf nutritional content, following plant defense literature which finds that leaves that are higher in nitrogen (and thus have a lower C:N ratio) are more nutritious and thus more palatable to herbivores (Mattson 1980, Agrawal and Fishbein 2006). We collected and dried one leaf per experimental plant in a drying oven for three days at 45°C. We then ground the dried leaf material into a smooth powder using a grinder and packaged approximately 2 to 5 mg of the samples in tin foil capsules. We used an ECS 4010 – CHNS-O Elemental Combustion system (Costech Analytical Technologies) to determine the carbon and nitrogen content per mass of sample for each species and *V. riparia* genotype. We used atropine as a standard with a known carbon and nitrogen content. The carbon to nitrogen ratio within the samples was calculated using the percent carbon and nitrogen contents supplied by the chromatograms, and the mass of the sample combusted.

Domatia

Domatia are plant-formed structures that shelter mutualistic mites, who in turn patrol the leaf surface eating fungus and microarthropods, thus serving as an indirect defense (Agrawal and Karban 1997, Norton et al. 2000, 2001, Romero and Benson 2004, 2005). In *Vitis*, these structures are formed out of leaf hairs that cluster in vein junctions creating a 'canopy' mites can nestle into and lay their eggs within. We scored size and hair density of four domatia on three dried, pressed leaves per experimental plant under a dissecting microscope. For size, we measured the radius of each domatium using a micrometer and scored the density of the hairs within the domatium on a nine point scale adapted from the OIV code O-085/U-33 scale used by plant breeders to assess leaf hair density (IPGRI et al. 1997). On our scale, a domatium density of zero represents a junction between veins that is devoid of hairs, while a score of nine represents a dense, full domatium with no leaf surface visible underneath. For leaf undersides that were fully pubescent, we were unable to assign a domatium radius measurement, as there is no discernable difference between domatium and laminar trichome cover. For other species, we multiplied the radius and the density score together to get a metric we term 'domatia index' (for figures showing the measurement strategy and more detail on the ranking system used, see Graham et al. 2023). Previous literature has established that domatia index is a strong predictor of mutualistic mite abundance, and thus the strength of the mutualistic defense, on grape leaf surfaces (Loughner et al. 2008).

Toughness

Leaf toughness is frequently cited in the literature as a direct defense against herbivory (Coley 1983, Malishev and Sanson 2015, Salgado-Luarte et al. 2023). To quantify the toughness of the leaves, we punctured three leaves per plant with a force gauge penetrometer. Because there was high noise in this toughness data we collected five measurements per leaf.

Trichomes

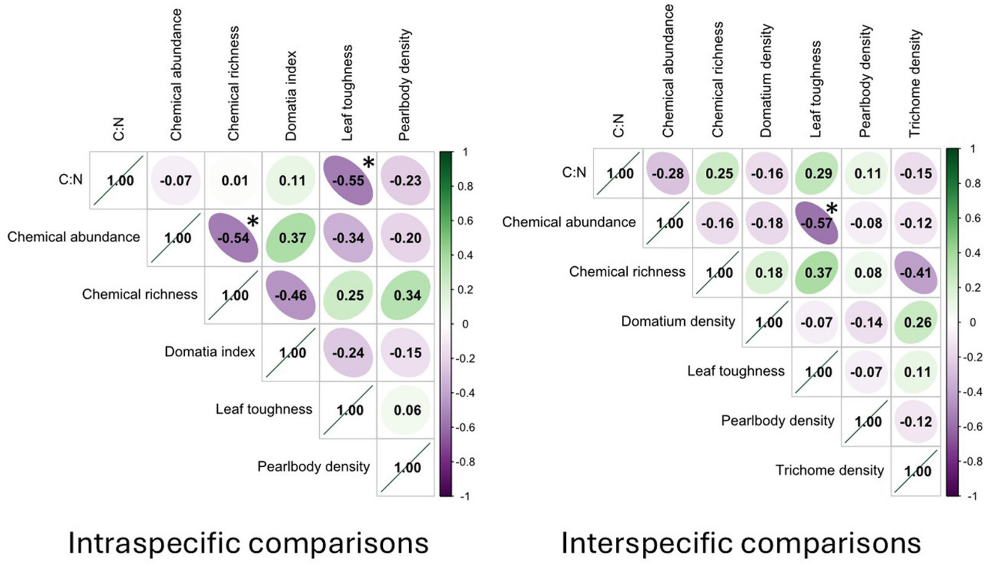
Trichomes are hair-like projections on the surface of plant tissue, which are hypothesized to serve a variety of purposes for plants, one of the most prominent of which is as a direct defense against chewing herbivores (Levin 1973, Karabourniotis et al. 2020, Fig. 2, Supporting information). For three dried, pressed leaves per plant, we characterized the density of trichomes on the abaxial leaf surfaces using the same nine point hair density scale used to measure domatium density.

Pearl bodies

Pearl bodies are microscopic orbs made of leaf epidermal cells that largely contain polysaccharides, proteins, and lipids (Paiva et al. 2009, Gerrath et al. 2015). These structures are primarily found on leaf undersides or on stems and have been hypothesized as a food source for plant mutualists such as mites and ants (Ozawa and Yano 2009, Paiva et al. 2009). We quantified the number of pearl bodies per leaf underside on fresh leaves using a dissecting microscope. We then standardized by leaf area to obtain the density of pearl bodies per centimeter squared of leaf surface.

Palatability

We characterized the palatability of grape leaf tissue to a generalist herbivore via a no-choice bioassay as a metric of the overall direct defense capacity of a plant. We used third instar *Spodoptera exigua* larvae, a generalist lepidopteran pest known to feed on grape (Merkx-Jacques et al. 2008). We placed a single larva on a grape leaf (mean = 3.3 leaves per *Vitis* species or genotype) in a closed cup and allowed it to feed for six days. At the end of this time period, we removed the leaf, assessed the survival of the herbivore, and scanned the leaf. We quantified the area of leaf tissue removed from the leaf using ImageJ (Schneider et al. 2012), and converted this to mass removed using the average leaf mass per area metric calculated from the dry leaves used in the domatia/trichome/pearl body measurements.



Intraspecific comparisons

Interspecific comparisons

Figure 2. Intraspecific (left) and interspecific (right) correlations between putatively defensive traits in *Vitis*. Greener colors represent more positive correlations, whereas more purple colors represent stronger negative correlations. Numbers inside the ellipses are Pearson's correlations coefficients calculated for trait pairs. Variables that are significantly correlated are indicated with asterisks. Correlations were computed using PICs rather than raw trait values in the case of the interspecific comparisons to account for phylogenetic relatedness.

Environmental variables

We downloaded occurrence data for each *Vitis* species from the global biodiversity information facility (GBIF) database using the R package 'rgbif' (Chamberlain et al. 2012, GBIF.org 2023). We were unable to obtain locality data for two *Vitis* species (*V. treleasei* and *V. yenshanensis*) because they did not exist in the GBIF database. As a result, the climate analyses are conducted with 19 *Vitis* species rather than the full 21species listed in Fig. 1. We cleaned our dataset of occurrences in two ways. First, we filtered down the occurrence records to only those marked as being from research-grade preserved specimens. Then, we used the R package 'CoordinateCleaner' to remove occurrences around country capitals, the GBIF headquarters, and institutions of biodiversity research, as well as duplicate occurrences, occurrences with equal latitude and longitude coordinates, coordinates at country centroids, zeros, outliers, and coordinates within bodies of water (Zizka et al. 2020). This resulted in a total of 16 641 occurrences for the species in this study.

We did not have precise locality of origin information available in the USDA-ARS germplasm database; for most accessions, the most precise location available was at the state or province level. For accessions that did have location data available, we manually verified that the collection location fell within the range of species occurrences obtained from GBIF. To obtain occurrence points for *V. riparia* genotypes, we binned the 1748 research-grade occurrences for *V. riparia* by state/province of origin and randomly sampled an occurrence from each state that our accessions originated from. Rather than using the state centroid, this approach produced an in-state occurrence point from habitats with confirmed *V. riparia* presence (Supporting information).

Using this cleaned set of occurrences, we then extracted climate data for each latitude/longitude coordinate from the WorldClim database using the R package 'raster' (Fick and Hijmans 2017, Hijmans 2022). We calculated mean location coordinates and environmental variable values for each of the 21 *Vitis* species used in this study (Supporting information).

To test for patterns of defense trait expression consistent with plant defense theory, we narrowed down the available GBIF and WorldClim data to four metrics that we expect to correlate with defense traits according to the LHDH and RAH: absolute value of latitude (a measure of distance from the equator), temperature seasonality (bio4), annual precipitation (bio12), and precipitation of the driest month (bio14). In our analyses, tests of the latitudinal herbivory defense hypothesis (LHDH) are made by comparing defense traits and latitude or seasonality. We include either latitude or seasonality in this hypothesis testing because, while correlated, we expect that temperature seasonality is representative of the relative growing season length that plants are exposed to herbivory, and thus may be expected to correlate with defense investment in ways that are distinct from latitude alone. Tests of the resource availability hypothesis (RAH) are made by comparing defenses and metrics of precipitation because aridity is an environmental stressor predicted (though not always found) to impact plant defense (Coley et al. 1985, Koricheva et al. 1998, Pearse and Hipp 2012).

Statistical analyses

All analyses were performed using R ver. 4.4.1 (www.r-project.org). We computed species/genotype means for each trait and environmental variable investigated. To test for intraspecific correlations between traits via the Pearson method, we used the *cor* and *cor.test* functions in base R on trait pairs, and constructed correlograms using the function *corrplot* in the R package 'corrplot' ver. 0.94 (Wei and Simko 2024). To test whether features of the abiotic environment predict the defense trait investment of *V. riparia* genotypes, we ran separate linear models for each environmental variable with that variable as the predictor and defense trait values as response variables. We chose not to combine the environmental predictors into one model for two reasons: the predictor variables are correlated with each other, and thus it would be statistically inappropriate to include them together in the same model, and because we were interested in teasing apart the distinct relationships between different facets of the abiotic environment and defense evolution rather than combine them into a less interpretable metric.

To test for correlations between defense traits across *Vitis* species, we corrected for non-independence using phylogenetic independent contrasts (PICs). PICs were computed using the *pic* function in the R-package 'phytools' (Revell 2024). This required a *Vitis* phylogenic tree pruned to the species in this study, which we sourced from (Graham et al. 2023). We then used the 'cor.test' function to test for evolutionary correlations between the phylogenetically corrected trait values. To test whether there is evidence for the LHDH or the RAH across *Vitis* species, we used the phylogenetic generalized least squares (PGLS) method implemented through the function *gls* in the R-package 'nlme' ver. 3.1-166 using a Brownian motion correlation structure with environmental variables as predictor variables and defense traits as response variables (Pinheiro et al. 2025). We ran these models separately for each environmental predictor the same reasons described in the above paragraph. Because each test represents a distinct hypothesis, we did not correct for multiple comparisons (Streiner 2015, Rubin 2021). Specifically, we treat each a priori hypothesis as distinct, predicting that traits would assort into defense syndromes, and that variation in defense traits would be tied to proximity to the tropics and resource availability. While correcting for multiple comparisons can reduce the likelihood of falsely rejecting a null hypothesis, it can also obscure biologically meaningful trait relationships. As a result, we follow the statistical advice of Streiner (2015) and Rubin (2021) and present uncorrected p-values for each hypothesis with an associated linear relationship in the Supporting information.

In order to assess whether defense traits measurements from *Vitis* vines cluster into groups of plants with similar trait values, which would be indicative of plant-defense syndromes, we additionally performed a principal components analysis (PCA) using plant-level defense trait means at both the intraspecific and interspecific levels. This was implemented via the *prcomp()* function in base R.

Results

Intraspecific trait–trait correlations

At the intraspecific level, we observed only two significant correlations between defense traits (Fig. 2). Tougher leaves had a lower carbon to nitrogen ratio, meaning that nitrogen makes a greater contribution to the mass of the leaf in tougher *Vitis* leaves (p = 0.0115). Additionally, the richness of metabolites in leaf samples was negatively correlated with the abundance of those metabolites (p = 0.0300). According to this result, leaves with a higher number of metabolites have less metabolite investment overall, perhaps demonstrating a tradeoff between chemical defense strategies. No *V. riparia* genotypes exhibited trichomes on their leaf surfaces outside of domatia, so we dropped this trait from the intraspecific analyses. Principal component analysis revealed a lack of clustering of defense trait values at the intraspecific level, providing no evidence of coherent defense syndromes (Supporting information).

Intraspecific trait–environment correlations

In our test of the latitudinal herbivory-defense hypothesis, we detected intraspecific trait–environment relationships between leaf toughness and latitude (p = 0.0118, Fig. 3a, Supporting information) as well as between leaf C:N ratio and latitude (p = 0.000147, Fig. 3b, Supporting information). The direction of these effects reveals that *Vitis riparia*

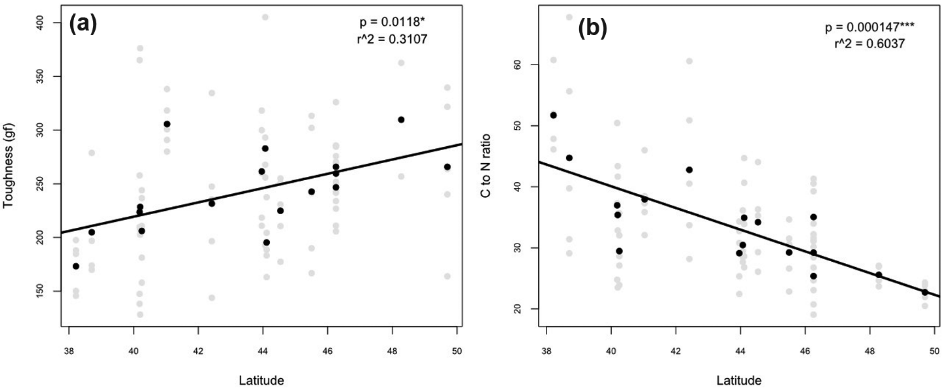


Figure 3. Contrasting correlations between defense traits and latitude within *Vitis riparia*. Black points represent genotype means, while grey points depict plant level means. Trendlines represent linear model estimates for the relationships between defense traits and latitude.

genotypes further from the equator have more tough, but less nitrogen-rich leaves. Leaf C:N content also correlated with temperature seasonality, where environments with more seasonal temperature variation have lower carbon-to-nitrogen ratios ($p = 0.0136$). This means that *V. riparia* genotypes in more variable regions have a greater proportion of nitrogen in their leaves than those native to regions with longer growing seasons, which corresponds with the expectations of the latitudinal herbivory-defense hypothesis. However, we did not detect a significant correlation between leaf toughness and seasonality ($p = 0.577$). We present all effect sizes, degrees of freedom, and p-values for intraspecific linear relationships between traits and latitude in the Supporting information. We found no support for the hypothesis that resource availability in the form of precipitation predicts investment in defense traits across *V. riparia* genotypes.

Interspecific trait–trait correlations

At the across-species (interspecific) scale, we also saw few trait-trait correlations (Fig. 2). However, we did detect a significant negative correlation between secondary metabolite abundance and leaf toughness ($p = 0.00841$), which is in line with a tradeoff between physical and chemical defense across species. For a depiction of how our measured traits vary across the *Vitis* phylogeny, see the Supporting information. Again, we saw no evidence of clustering of defense trait values across species via PCA that would be clear evidence of defense syndromes; instead PC values are relatively evenly distributed across trait space (Supporting information). Plant-level PCA scores for traits did cluster with other points from plants of the same species (see colored hulls in figure). The traits that contribute the most to PC1 include trichome density, C:N ratio, metabolite richness, domatium hair density, and pearl body density, with higher values of PC1 corresponding to greater trichome and domatium density and lower chemical richness, C:N, and pearl body density. One could interpret this as indicating a trend toward species such as *V. girdiana* and *V. cinerea* investing in trichome-related defenses, while the species *V. monticola*, *V. treleasei*, and *V. nesbittiana* occupy areas of the trait space representative of greater investment in chemical richness, high C:N, and pearl body density. Interestingly, *V. treleasei* and *V. girdiana*, which do not overlap in trait space, are sister to each other in our phylogenetic tree. In case of PC2, higher values correspond to greater investment in chemical abundance, and lower investment in leaf toughness and trichomes, suggesting the existence of alternate chemical or physical defense strategies. Thus, while clear separation of species in defense syndromes is not supported by our correlational data, the interspecific PCA does indicate that some *Vitis* species to bear similarities to each other in defense expression.

Interspecific trait–environment correlations

We found mixed support for the LHDH, and no support for the RAH, across *Vitis* species. Pearl bodies ($p = 0.0273$, Fig. 4a, Supporting information) and C:N ratio ($p = 0.0299$, Fig. 4b, Supporting information, note that while significant, the model where latitude predicts the investment in C:N does not have strong predictive power) correlate negatively with latitude, while chemical abundance correlates with

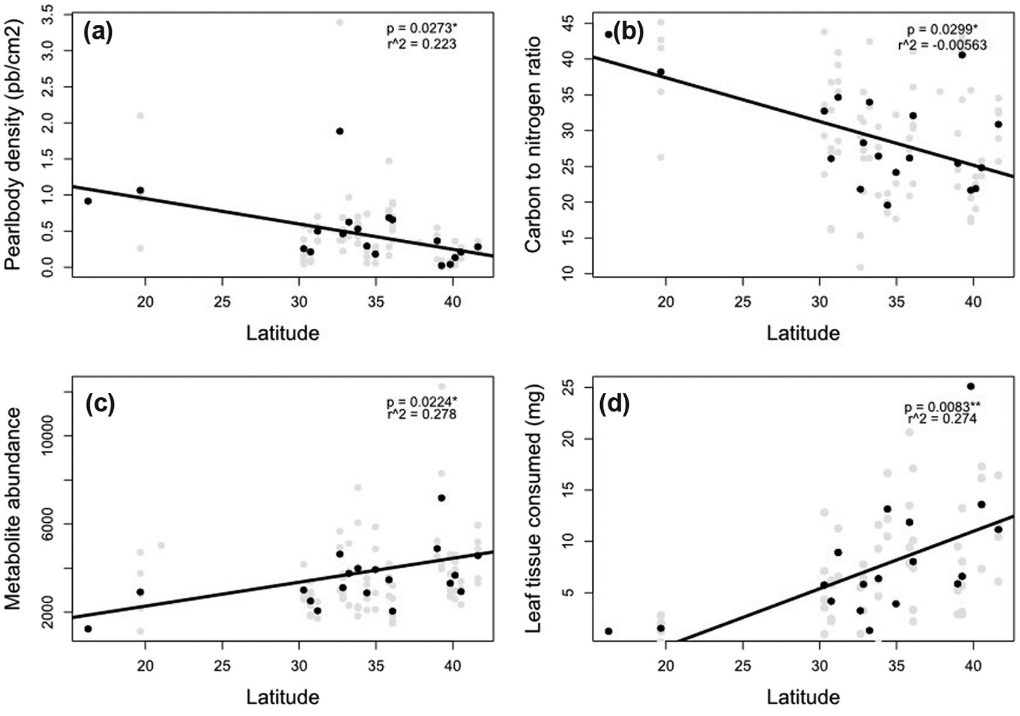


Figure 4. Interspecific relationships between defense metrics and species latitude. All relationships are significant at the $\alpha = 0.05$ level. Black points represent species means. Grey points are plant-level averages for the trait.

latitude positively (p = 0.0224, Fig. 4c, Supporting information). These first two observations are in line with our prediction that more equatorial environments have more defended, less palatable plant species. However, secondary metabolite abundance shows the opposite pattern with latitude, where more northern environments have more abundant metabolites. Carbon-to-nitrogen ratio was also negatively correlated with latitude at the intraspecific level, suggesting that this relationship persists across scale. Only metabolite abundance correlated with seasonality, again positively (p = 0.0162, Supporting information). Additionally, we detected a significant relationship between herbivory, a metric of overall plant defense, and the latitude of the home environments of *Vitis* species (p = 0.0083, Fig. 4d, Supporting information), whereby the generalist herbivore in our bioassay ate less leaf material from plants that originate closer to the equator. We present all effect sizes, degrees of freedom, and p-values for linear interspecific relationships between traits and latitude in the Supporting information. As seen at the intraspecific scale, no defense traits correlated with annual precipitation or precipitation of the driest month across *Vitis* species.

Discussion

We used a phylogenetic comparative framework and a powerful common garden experimental design to test the scale-dependance of plant defense syndromes and trait-environment correlations in the wild grape genus *Vitis*. Because the common garden method controls for trait plasticity, any significant relationships detected implies that evolution has shaped present-day variation in these traits. Our results demonstrate that strong correlations between *Vitis* defense traits, especially those indicative of synergism, are rare both across and within species, and we thus conclude that these traits largely evolve independently of each other. Indeed, instead of sorting into predictable defense syndromes, we saw relatively more frequent significant relationships between traits and the abiotic environment, specifically latitude of origin and seasonality.

Trait–trait correlations across scales

The general lack of significant correlations between traits at either level of biological organization within this study stands in contrast to plant-defense theory that predicts that plants should exhibit complex arrays of positively or negatively covarying defense traits. Syndromes have long fascinated plant biologists and garner some support in experimental tests of defense trait expression (Agrawal and Fishbein 2006, Johnson et al. 2014, Farias et al. 2020, Zhao et al. 2021). Principal component analysis at the interspecific level did suggest that some *Vitis* species express similar principal component values summarizing variation in traits (i.e. they overlapped in trait space with some species but not others), but this pattern did not translate to significant positive trait–trait correlations, and was not echoed at the intraspecific level. Thus, we detected little support for trait–trait synergisms at either scale within this study, instead observing solely negative correlations between traits. This echoes the findings of Moles and colleagues (2013), where only five out of 45 pairwise correlations between physical and chemical defense traits collected across a broad array of plant taxa were significant, and with three of the five pairs exhibiting tradeoff patterns. Similarly, while Johnson et al. (2014) found that 26 species of evening primroses exhibited covariation between traits primarily indicative of tradeoffs. This inconsistency of the syndromes across taxa underscores the importance of incorporating multiple scales into investigations. Indeed, all of the investigations of plant defense syndromes that we are aware of to date are tested at the interspecific scale, meaning that interesting within-species trait–trait interactions are likely being missed.

Recent theory suggests that correlations that are due to genetic or physiological constraint are more likely to be detectable at the intraspecific scale but break down at the interspecific scale, while correlations between traits that result from niche specialization (e.g. competitive/stress-tolerant/ruderal strategies, plant apparency) may be more likely to be observed across species (Agrawal and Hastings 2019, Agrawal 2020, Zhou et al. 2022). One reason for this is that life-history strategies such as growth form or fast growth are likely to be fixed at the species level, producing little variation within species (Futuyma 2010). Zhou and colleagues (2022) suggest that evaluating intraspecific correlations between traits is a more direct assessment of physiological or evolutionary constraint than assessing correlations across species, which may be complicated by environmental drivers of trait investment. Our results are in support of this theory, where the correlation between leaf toughness and leaf carbon:nitrogen content, likely a biproduct of plant physiology, is strong within but not across species. Additionally, our results suggest that investing in chemical defense may be at the expense of one form of physical defense (i.e. a tradeoff pattern), as we observed a negative relationship between leaf toughness and metabolite abundance across but not within species. We did not find evidence of the oft hypothesized tradeoff between direct and indirect defense traits across nor within species, a pattern which has received mixed support in previous investigations of defense investment in *Vitis* (English-Loeb and Norton 2006, Graham et al. 2023).

Why might trajectories of defense trait evolution shift across spatial or biological scales? One reason may be that the geographic ranges of populations within a single species are likely to be much smaller than the ranges of multiple species within a genus. Thus, the area occupied by a single species may not encompass enough of a gradient in resources or herbivore communities to impose constraints leading to a tradeoff between traits in a single species (Hahn and Maron 2016). Indeed, our intraspecific comparisons were conducted in *V. riparia*, which, while having a range that is much larger than that of many other wild grape species, still has a much more limited geographic range than the genus as a whole. As our common garden design should eliminate variation in trait expression due to plasticity, we expect that a lack of variation in defense strategies between populations produced the broad lack of trait correlations at the intraspecific scale observed in this study.

Conversely, as observed by Agrawal and Hastings (2019), resource allocation trade-offs that are present within species may be masked by variation in resource acquisition across species (see also van Noordwijk and de Jong 1986). A theoretical example used to explain this phenomenon is the 'car–house paradox,' which proposes that within any one household (i.e. within species), financial constraint means that owners must choose between spending more of their money on either a house or a car, but across households (i.e. across species), there is enough variation in financial acquisition that there is generally a trend that homeowners with more expensive houses tend to own more expensive cars (van Noordwijk and de Jong 1986, Arnold 1992). For this reason, we may be more likely to observe tradeoffs within species, but positive correlations across species. While we did observe tradeoffs between traits at the intraspecific level, we also observed a different negative correlation between chemical abundance and leaf toughness at the interspecific level, and no positive correlations between traits. If the intraspecific correlations between chemical abundance and richness and between leaf toughness and carbon-nitrogen content are the result of resource limitation, the lack of these tradeoff patterns at the interspecific level might suggest that variation in resource acquisition across species might be producing this pattern.

While we did not find evidence of plant defense syndromes in wild grape, there are likely aspects of defense against herbivory in the grape genus that we may be missing by virtue of only measuring select traits. For example, we did not intentionally induce defense traits in this study, meaning that we did not address the often predicted tradeoff pattern between constitutive and induced defenses (Karban and Myers 1989, Brody and Karban 1992, Koricheva et al. 2004, Agrawal and Hastings 2019). Because we sampled defenses one at a time with week-long intervals between collection, we do not think that our sampling caused measurable induction of defensive chemicals or physical traits. However, it is possible that some induction of defenses was caused by removal of plant tissue. Other defense-related traits to investigate in *Vitis* in the future might include calcium oxalate phytoliths or volatile chemicals that attract natural enemies, though the function of the former trait has not been well established in *Vitis* (Arnott and Webb 2000, Schwab and Wüst 2015).

Trait–environment correlations across scales

The LHDH, an offshoot of the more all-encompassing latitudinal biotic interactions hypothesis, has captivated and puzzled evolutionary ecologists for decades (Anstett et al. 2016, Baskett and Schemske 2018, Baskett et al. 2020). To our knowledge it has not been tested at multiple levels of biological organization simultaneously. Our findings demonstrate mixed support for its prediction that plants at more equatorial latitudes will be more defended than plants in temperate climes. We found that one form of indirect defense (pearl bodies) was more prevalent at lower latitudes, and that plants in these regions also have less nutrient-dense leaves (i.e. have a higher C:N). The former finding compliments a growing body of accounts of higher investment in indirect defense that provide food to bodyguard mutualists at more equatorial latitudes (Keeler 1980, Oliveira and Leitao-Filho 1987, Fiala and Linsenmair 1995). For example, latitude, present-day annual temperature, and precipitation were found to be strong predictors of the diversity of EFN- and ant domatia-bearing flowering plants (Luo et al. 2023). We also detected this negative correlation between latitude and C:N across *V. riparia* genotypes, suggesting that this evolutionary trajectory is repeated across biological scales. In contrast, *Vitis* species in more temperate climes had greater investment in secondary metabolite abundance. It is interesting to note that when we use temperature seasonality as the predictor in our trait–environment models in the place of latitude, only two correlations are significant (one at the intraspecific scale and one at the interspecific scale; Supporting information). Though latitude and temperature seasonality are highly correlated, seasonality does not vary 1:1 with latitude. Thus, we might predict that some other unmeasured feature of the environment that is influenced by latitude (e.g. herbivore pressure) is more important in shaping defense investment in and across *Vitis* species. Indeed, the observation that different traits correlate with latitude in opposite directions within *V. riparia* may suggest a niche-specialization tradeoff, whereby different herbivore communities in lower versus higher latitude environments have selected for different defense strategies in grape species. Evolutionary biologists often expect that specialization in diet increases towards the equator, but investigations of patterns in the specialization of insects on host plants do not always report consistent correlations with latitude (Salazar and Marquis 2012, Anstett et al. 2014). Though latitudinal characterizations of herbivore community on *Vitis* species are rare, one study did find that more northern species actually had a higher incidence of attack by the specialist grape phylloxera *Daktulosphaira vitifoliae*, a pattern largely driven by the susceptibility of *V. riparia* to the herbivore.

We also observed that *Vitis* species seem to be better defended overall closer to the equator, as the generalist herbivore *S. exigua* consumed less leaf tissue from grape species native to Central America than from species native to more northerly regions of America. Because our grape leaves were not defended by mutualist bodyguards during our bioassay experiment, it is unlikely that pearl bodies contributed to this trend. Plant nutritive value may be implicated in this pattern, but we did not see a direct effect of C:N ratio on herbivore performance. Thus, we may interpret our results to mean that no one defense strategy is the magic bullet to defend against *S. exigua* in this controlled setting, but instead some un-tested combination of defenses reduces their feeding. Additional tests factorially combining distinct defense traits would then be helpful to evaluate which combinations of traits impact herbivory in *Vitis* species.

A recent meta-analysis of papers exploring biogeographical patterns of ant–plant mutualism found that such mutualisms are more effective at improving plant performance in arid environments, presenting evidence of an advantage to investing in indirect defense in low precipitation environments (Leal and Peixoto 2017). Anecdotally, *V. arizonica*, a species of grape native to the southwestern United States, experiences some of the lowest annual rainfalls in our dataset, and also produces huge quantities of pearl bodies (Supporting information). However, our overall results suggest that precipitation is not a significant driver of defense evolution within nor across *Vitis* species. It is important to note that the *Vitis* vines used in this experiment were not grown in nutrient scarce environments that would mimic what they encounter in their home ranges. Instead, the plants were grown in a common garden environment and thus all plants were subjected to the same environmental conditions. It is possible, therefore, that the vines would demonstrate plasticity in their defense phenotypes if sampled in their natural ranges, and correlations between traits and environmental variables might thus be observed. Though we did not find evidence that environmental stress due to precipitation is a determinant of *Vitis* defense investment, water is but one resource that plants need for growth. Other tests of the impacts of resource availability have considered essential nutrients such as nitrogen and even light availability and have found that different resources may or may not correlated with particular plant defenses (Koricheva et al. 1998, Endara and Coley 2011).

Conclusions

Using a powerful pairing of common garden trait quantifications and a comparative phylogenetic framework we demonstrated a lack of hypothesized correlations between defense traits both across and within *Vitis* species. Instead, latitude was a strong predictor of plant defense investment at both levels of biological organization, although trait–latitude correlations shifted across scales and traits. This work thus challenges the oft-hypothesized tradeoff and synergism framework for defense trait evolution in plants, and demonstrates the scale dependency of hypotheses that related this defense evolution to the abiotic environment. Future work on plant defense evolution should carefully consider the relevancy of plant defense hypotheses at different geographic and biological scales.

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Author contributions

Carolyn D. K. Graham: Conceptualization (equal); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (equal); Project administration (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (equal). **Marjorie G. Weber**: Conceptualization (supporting); Funding acquisition (lead); Methodology (equal); Supervision (equal); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.4qrfj6qp1 (Graham and Weber 2025).

Supporting information

The Supporting information associated with this article is available with the online version.

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