Multiple metrics of latitudinal patterns in insect pollination and herbivory for a tropical-temperate congener pair

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Abstract. The biotic interactions hypothesis posits that biotic interactions are more important drivers of adaptation closer to the equator, evidenced by "stronger" contemporary interactions (e.g., greater interaction rates) and/or patterns of trait evolution consistent with a history of stronger interactions. Support for the hypothesis is mixed, but few studies span tropical and temperate regions while experimentally controlling for evolutionary history. Here, we integrate field observations and common garden experiments to quantify the relative importance of pollination and herbivory in a pair of tropical-temperate congeneric perennial herbs. *Phytolacca rivinoides* and *P. americana* are pioneer species native to the Neotropics and the eastern United States, respectively. We compared plant–pollinator and plant–herbivore interactions between three tropical populations of *P. rivinoides* from Costa Rica and three temperate populations of *P. americana* from its northern range edge in Michigan and Ohio. For some metrics of interaction importance, we also included three subtropical populations of *P. americana* from its southern range edge in Florida. This approach confounds species and region but allows us, uniquely, to measure complementary proxies of interaction importance across a tropical-temperate range in one system. To test the prediction that lower-latitude plants are more reliant on insect pollinators, we quantified floral display and reward, insect visitation rates, and self-pollination ability (autogamy). To test the prediction that lower-latitude plants experience more herbivore pressure, we quantified herbivory rates, herbivore abundance, and leaf palatability. We found evidence supporting the biotic interactions hypothesis for most comparisons between *P. rivinoides* and north-temperate *P. americana* (floral display, insect visitation, autogamy, herbivory, herbivore abundance, and young-leaf palatability). Results for subtropical *P. americana* populations, however, were typically not intermediate between *P. rivinoides* and north-temperate *P. americana*, as would be predicted by a linear latitudinal gradient in interaction importance. Subtropical young-leaf palatability was intermediate, but subtropical mature leaves were the least palatable, and pollination-related traits did not differ between temperate and subtropical regions. These nonlinear patterns of interaction importance suggest future work to link interaction importance to climatic or biotic thresholds. In sum, we found that the biotic interactions hypothesis was more consistently supported at the larger spatial scale of our study.

Key words: biogeography; biotic interactions hypothesis; herbivory; latitudinal gradient; palatability; plant defense; plant–insect interactions; pollination; species interactions.

Introduction

The biotic interactions hypothesis states that biotic interactions are more important drivers of adaptation in tropical than temperate regions and contribute to high tropical biodiversity (Dobzhansky 1950, Fischer 1960, Pianka 1966, Schemske 2009, Schemske et al. 2009). Following Schemske (2009), interaction importance is defined as the proportion of variation in fitness in a population caused by a biotic interaction, so that the abiotic environment causes increasingly more variation in fitness as latitude increases, and biotic interactions cause increasingly more variation in fitness as latitude decreases. Quantifying the relative contribution of all potential components of the abiotic and biotic environments to variation in fitness is hardly feasible in even

one population. Thus, biotic interaction importance is typically approximated in one of three ways (Schemske et al. 2009): latitudinal comparisons of interaction "strength" (e.g., caterpillar predation rate; Roslin et al. 2017), interaction prevalence (e.g., proportion of plants with endophytic fungi; Arnold and Lutzoni 2007), or phenotypic traits involved in interactions (e.g., shell defense of marine mollusks; Palmer 1979). Many plant mutualisms have been shown to be more prevalent in tropical communities, such as animal pollination (Regal 1982, Rech et al. 2016), animal seed dispersal (Moles et al. 2007), ant–plant interactions involving extrafloral nectaries and domatia (Coley and Aide 1991, Davidson and McKey 1993), mycorrhizal fungi (Delavaux et al. 2019), and endophytic fungi (Arnold and Lutzoni 2007). Measuring prevalence of an interaction in a community is relatively straightforward, but comparisons of interaction strength or traits related to interactions are more challenging, and thus the approaches and conclusions have been more controversial (Schemske et al. 2009, Anstett et al. 2016, Dyer and Forister 2019). In particular, latitudinal patterns in herbivore pressure, plant defense, and pollination specialization have been contested, with no clear resolution (Moles et al. 2011a, Ollerton 2012, Moles 2013, Moles and Ollerton 2016). Here, we address some of the long-standing limitations and gaps in previous work on insect pollination and herbivory, important plant–animal interactions that are still poorly understood with respect to latitudinal patterns. In addition to testing the biotic interactions hypothesis, studying the causes and consequences of geographic variation in biotic interactions contributes to our general understanding of context-dependency of biotic interactions (Thompson 2005, Bronstein 2015, Weber et al. 2017).

Comparisons of latitudinal trends in biotic interaction importance are generally conducted at one of three scales, each with advantages and disadvantages: (1) among populations of a wide-ranging species, (2) among species of a wide-ranging genus, or (3) across communities at multiple latitudes that differ in species composition. Focusing on a wide-ranging species allows for quantifying detailed metrics of interaction importance, e.g., by quantifying secondary metabolites with known defensive function or by measuring herbivory rates in the field. This approach is commonly used to study plant–herbivore interactions (Pennings and Silliman 2005, Salgado and Pennings 2005, Anstett et al. 2014, 2015, Baskett and Schemske 2018), but has found mixed support for the biotic interactions hypothesis (Moles et al. 2011a). One of its major limitations is that species' ranges are limited (usually to one biome) relative to the scale of the biotic interactions hypothesis, and most studies have focused on the temperate zone, typically spanning 15° (Anstett et al. 2016). This range may be problematic because the biotic interactions hypothesis is a comparison of temperate and tropical communities (Dobzhansky 1950), and it remains unclear whether interaction importance should scale linearly with latitude, or change abruptly at climatic thresholds that may only be captured by large-scale studies (Moles et al. 2007, 2011a, Kozlov et al. 2015a, Anstett et al. 2016, Baskett and Schemske 2018). For example, a meta-analysis found that the latitudinal diversity gradient is stronger in studies that cover a wider latitudinal range, although latitudinal midpoint did not affect the strength of the gradient (Kinlock et al. 2018). Furthermore, wide-ranging species are a nonrandom subset of all species, and attributes associated with large ranges could impact biotic interactions and our interpretations of studies of the biotic interactions hypothesis. For example, the biotic interactions hypothesis would predict more reliance on animal pollinators at lower latitudes, so selfing rate would be predicted to positively correlate with latitude in a plant with mixed mating. However, self-pollination was associated with larger ranges in a sister species comparison (Grossenbacher et al. 2015), so a latitudinal pattern in selfing rate in wide-ranging species could be swamped by selection for a high selfing rate across the range.

Studies of wide-ranging genera can address some of the limitations of one-species studies by spanning a wider geographic range than any one species and including species with different range sizes. Their shared evolutionary history allows for comparisons of shared traits such as secondary metabolites with known defensive function (Rasmann and Agrawal 2011, Pearse and Hipp 2012, Anstett et al. 2018). However, measuring interaction importance across a genus trades breadth for detail, as measuring interactions in the field across many species is infeasible. This is unfortunate, as traits and ecological interactions in the field contextualize each other; for example, one might find that different levels of defense are needed to reduce herbivory to similar levels in different populations, or that populations with similar defenses experience very different levels of herbivory. Quantifying herbivory tolerance would provide further context to interpreting patterns of herbivory and defense (Stowe et al. 2000), but studies of latitudinal patterns in herbivory tolerance are rare and limited to single-species studies (Wieski and Pennings 2014, Lehndal and Ågren 2015b). Furthermore, using species mean trait values for within-genus comparisons may obscure signals of latitudinal patterns within species. For example, observed trends across *Asclepias* of more toxic, diverse, and inducible cardenolides at low latitudes (Rasmann and Agrawal 2011) contrasts with greater herbivore resistance observed in higher-latitude populations of *Asclepias syriaca* (Woods et al. 2012). The opposite pattern is seen in *Oenothera*, with stronger chemical defenses at higher latitudes across the genus (Anstett et al. 2018), but at lower latitudes within *O. biennis* (Anstett et al. 2015). In both of these systems, the conflicting results at different scales are complicated by the breadth–depth trade-off, as the single-species studies used herbivory in common outdoor gardens to quantify resistance and identify

important defensive traits, while the genus-level studies compared secondary metabolites that are typically defensive in the genus, without the context of herbivore pressure, other defensive traits that could be important in each species, or herbivory tolerance. Although the biotic interactions hypothesis does not specify a taxonomic scale, presumably it should apply both within and across species (Anstett et al. 2016). If discrepancies are common, further research is needed to discover whether the causes are methodological or biological.

In contrast to studies within species or genera, community surveys can span wide geographic scales and encompass a diversity of abiotic environments and taxonomic groups, so biases of focusing on temperate biomes and/or wide-ranging taxa can be avoided (Adams et al. 2009, Moles et al. 2011b, Kozlov et al. 2015b, Lim et al. 2015, Moeller et al. 2017). However, community sampling has drawbacks; for example, we lack "common currencies" of traits to compare plant defenses between unrelated species (Agrawal and Weber 2015), and species can arrive at similar outcomes in interactions using rather different traits (Agrawal and Fishbein 2006). Recent advances in metabolomics may allow large-scale community comparisons of chemical defense (Sedio et al. 2018), though defensive function remains difficult to demonstrate across many species. Additionally, communities sampled at large geographic scales will have mostly separate evolutionary histories. For example, Moles et al. (2011b) compared various putatively defensive traits in ~300 species of herbs, grasses, trees, lichens, and vines across 85 plant families, with a median of one species per family. Furthermore, the most diverse terrestrial communities, wet lowland tropical forests, are often poorly represented in community-level studies (Olesen and Jordano 2002, Moles et al. 2011b, Kozlov et al. 2015b). Since the biotic interactions hypothesis is focused on explaining the astonishingly high biodiversity of tropical ecosystems, under-sampling these ecosystems may be missing unique tropical phenomena. Similar to genus-level studies, collecting detailed interaction data is infeasible at large geographic and taxonomic scales. For example, estimating herbivory from leaf litter is a high-throughput approach to randomly sample forest communities (Adams et al. 2009), but cannot capture complete leaf consumption, which can be a substantial fraction of herbivory (Lowman 1984, Baskett and Schemske 2018). While latitudinal patterns in herbivory and plant defense have been studied using approaches at multiple scales (Anstett et al. 2016), most latitudinal comparisons of plant–pollinator interactions thus far have been at the community level (Rech et al. 2016, Moeller et al. 2017).

Here, we bridge gaps (in both advantages and limitations) between the detailed single-species studies limited to smaller latitudinal ranges, and multi-species surveys that can cover large latitudinal ranges but cannot thoroughly quantify multiple, interdependent components of biotic interaction importance. We combine species- and genus-level sampling by quantifying species interactions in depth in tropical and temperate regions, using a pair of closely related, ecologically similar species. Unlike a typical genus-level comparison, we focus on only two species, which confounds species identity and region. However, limiting our study to a small number of species allows for great detail, and we quantify several ecological and evolutionary proxies of the importance of herbivory and pollination, including rarely measured factors such as leaf age. Our study spans 32° latitude, comparing the Neotropical *Phytolacca rivinoides* (Phytolaccaceae) in Costa Rica to its congener *P. americana* at its southern and northern range edges in the USA. For both herbivory and pollination, we pair in situ field studies evaluating interaction strength over multiple seasons with phenotypic trait measurements in a common garden. Few studies have examined latitudinal patterns for multiple biotic interactions in a single system, so this study allows us to compare results for a mutualistic and an antagonistic interaction.

According to the biotic interactions hypothesis, we predict that pollination and herbivory rates will be greater and that traits will show signatures of stronger selection by herbivores and pollinators at lower latitudes. These predictions assume that pollination and herbivory have fitness consequences related to interaction strength, and that trait differences between populations are driven by natural selection. Specifically, we predict that (1) *P. rivinoides* has more attractive and rewarding flowers, receives more visits from insects, and has evolved a reduced ability to self-pollinate compared to *P. americana*, and (2) that *P. rivinoides* experiences greater herbivore pressure (higher herbivory rate and greater herbivore abundance) and has evolved to be better defended than *P. americana*. We predict that these various measures of interaction importance are intermediate for *P. americana* at its subtropical southern range edge compared to its northern range edge and to *P. rivinoides*, assuming that interaction importance decreases with latitude.

<center>METHODS</center>

<center>*Study system and sites*</center>

Phytolacca rivinoides Kunth & C.D. Bouché and *P. americana* L. are well-suited for a test of the biotic interactions hypothesis comparing tropical and temperate regions (Fig. 1). *Phytolacca rivinoides* is native throughout the Neotropics, from 16° S to 20° N (Murray 1988), and *P. americana* is native from subtropical to north-temperate eastern United States (27–44° N; Sauer 1952). Both species are bird-dispersed, short-lived, perennial herbs that rely on long-lived seed banks to colonize disturbed areas, where they are abundant and easily found (Sauer 1952, Murray 1988, Veldman et al. 2007). One ecological difference between the species that could potentially mediate fitness effects of pollination
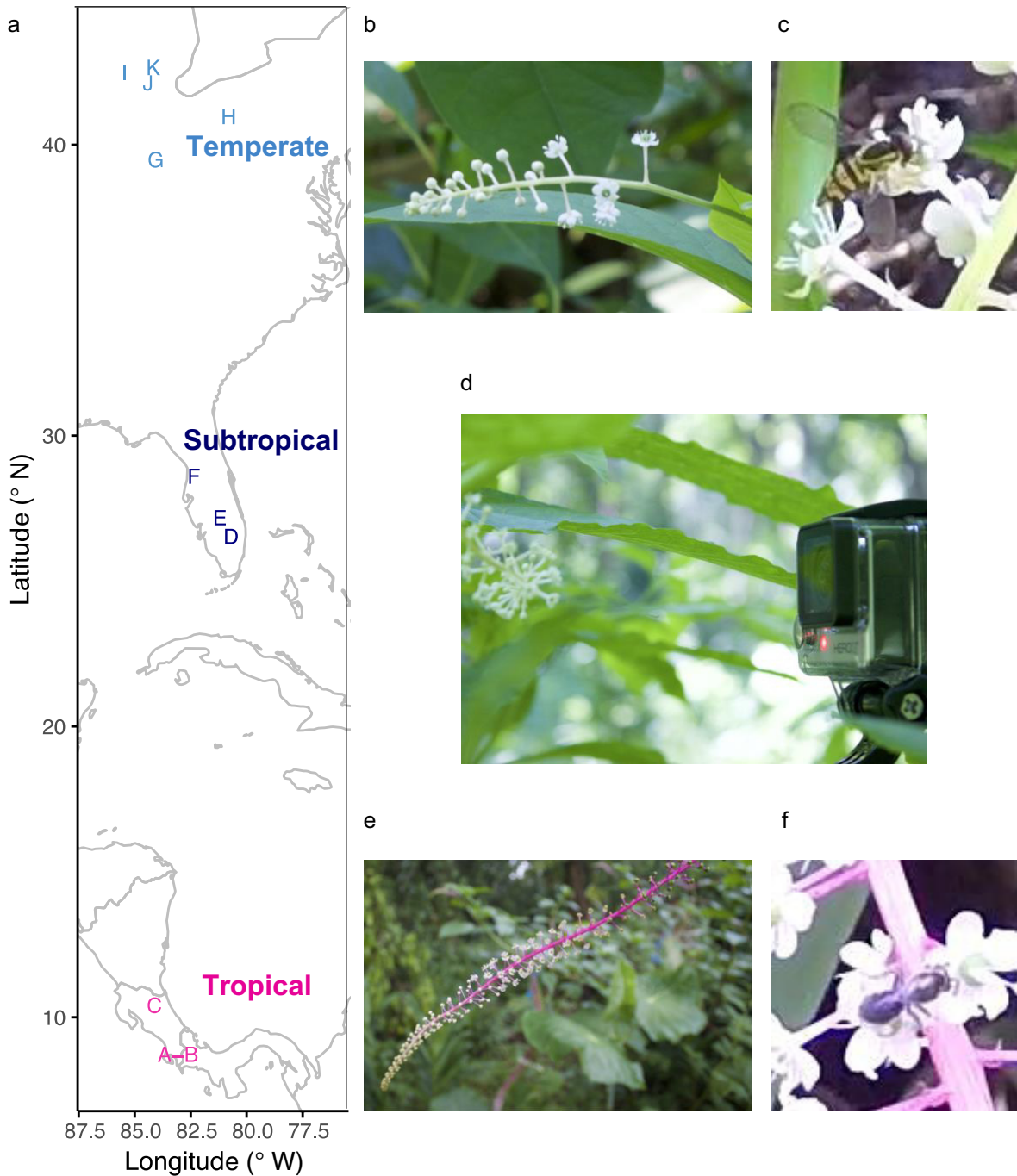
Fig. 1. Study species and sites. (a) Populations used for in situ studies of pollination and herbivory and/or sources of seeds for common gardens planted at Michigan State University. Populations are grouped throughout the paper into three consistently color-coded "regions": (b–d) temperate and subtropical *Phytolacca americana* and (e, f) tropical *P. rivinoides*. Letters in (a) correspond to Appendix S1: Table S1. Temperate populations G–I were used in common garden studies, while I–K were used for in situ studies. Labels for A and B would overlap, so we separated for clarity. (c, f) Example screenshots of video recordings used to estimate floral visitation rate (see also Video S1). (d) Camera positioning for video recording.

and herbivory is that *P. rivinoides* is semelparous, with a life span of about two years (Murray 1988), while *P. americana* is an iteroparous perennial, reemerging each spring from a large taproot. We found no reports of

a mean or maximum life span, but it can live at least 4 yr (Sauer 1952). The genus *Phytolacca* is monophyletic and contains 20 species, mostly in the New World tropics; relationships within the genus are poorly resolved, but it

is unlikely that our focal species are each other's closest relative (Ali et al. 2015). *Phytolacca americana* is the only member of the genus in most of its range (Sauer 1952). However, some authors have considered *P. americana* in Florida and the coastal southeastern United States to be a distinct subspecies (Sauer 1952) or even species (*P. rigida* Small) based on phenotypic differences: erect racemes during fruiting and shorter pedicels in Florida (Small 1905, Hardin 1964). Without further evidence of reproductive isolation and genetic differentiation, we treat subtropical populations as *P. americana*.

We evaluated species interaction importance using multiple field and common garden studies in three regions, which we refer to as tropical, subtropical, and temperate (Fig. 1a, Table 1; Appendix S1: Table S1): *P. rivinoides* in Costa Rica (8.7°–10.4° N), *P. americana* in Florida (26.5°–28.6° N), and *P. americana* in Michigan and Ohio (39.5°–42.7° N). All measurements of interaction importance were quantified in three populations in each region (Fig. 1a, Table 1; Appendix S1: Table S1). Some measurements were performed in all three regions, and others only in tropical and temperate regions. Note that two temperate populations differ between common garden and in situ studies (G-I and I-K from Fig. 1a, respectively). All populations were abutting or surrounded by natural vegetation in rural landscapes and had varying disturbance histories (Appendix S1: Table S1).

Prior to our studies, the pollination biology of these *Phytolacca* was unknown. Both species have racemes with many white flowers (6 mm diameter). Nectaries are tucked at the base of a green pistil. Anthers are dehiscent when flowers open, and tend to fall off the second day of flowering, but the period of stigma receptivity is unknown. Typically, *P. americana* has 5 tepals and 10 stamens and carpels (Hardin 1964), and *P. rivinoides* has 5 tepals and 16 stamens and carpels (Fassett and Sauer 1950).

In contrast to pollination, there is previous work on herbivory in *P. americana*, comparing herbivory rates, herbivore abundance, and plant defense (palatability) in multiple populations along a latitudinal gradient (Baskett and Schemske 2018). The purpose of that study was to describe the shape of latitudinal patterns, so populations between the temperate and subtropical regions of the present study were included. We found greater herbivore pressure and stronger defense at lower latitudes, with threshold patterns. Data from the palatability experiment in Baskett and Schemske (2018) are re-analyzed in the present study in the context of including tropical populations and breaking palatability down into survival and growth components. Field herbivory and herbivore abundance data were collected specifically for the present study.

Baskett and Schemske (2018) determined that lepidopterans are the primary herbivores of *P. americana*, and identified five consumers along a gradient from Michigan to Florida: *Disclisioprocta stellata* Guenée (Geometridae), *Spodoptera eridania* Stoll (Noctuidae), and three species that shelter in and consume young leaves: *Psara obscuralis* Lederer (Crambidae), *Asciodes gordialis* Guenée (Crambidae), and *Argyrotaenia velutinana* Walker (Tortricidae). *Spodoptera eridania* and *A. velutinana* are highly polyphagous (Summerland and Hamilton 1955, Robinson et al. 2010), while the other species have more restricted diets in host families closely related to Phytolaccaceae (Allyson 1984, Cuenoud et al. 2002, Robinson et al. 2010).

Statistical analyses common across studies

For simplicity, we present analysis details with the methods for each study. The following details of analysis are common to all mixed-effects models in the present study unless specified otherwise. We used the lme function in the nlme package in R version 3.3.3 (R Core Team 2014, Pinheiro et al. 2017) and parameters were estimated using restricted maximum likelihood. Variance was rarely homogeneous between populations, so we often estimated variance separately for each population (specified in each analysis). Models fit assumptions of normally distributed residuals. Where there was a significant fixed effect of region for analyses with three regions, we used Tukey's HSD test to compare regional means ($\alpha = 0.05$).

TABLE 1. Prediction and study design for each metric of the importance of pollination and herbivory.

Interaction or trait	Prediction: low-latitude plants have...	Regions compared	Place measured
Floral display and reward	larger display, more nectar	tropical *P. riv.*, subtropical *P. am.*, temperate *P. am.*	common garden
Floral visitation rate	higher visitation rate	tropical *P. riv.,* temperate *P. am.*	in situ
Self-pollination (autogamy)	lower autogamy rate	tropical *P. riv.*, subtropical *P. am.*, temperate *P. am.*	common garden
Herbivory rates	higher herbivory rate	tropical *P. riv.*, temperate *P. am.*	in situ
Herbivore abundance	higher herbivore abundance	tropical *P. riv.*, temperate *P. am.*	in situ
Plant defense (palatability)	less palatable leaves	tropical *P. riv.*, subtropical *P. am.*, temperate *P. am.*	common garden

Notes: P. riv.: *Phytolacca rivinoides*, native to the Neotropics; *P. am.*: *P. americana*, native to the eastern USA. Populations correspond to regions in Fig. 1a and Appendix S1: Table S1.

Plant–pollinator interactions

We predicted that plants at lower latitudes invest in larger floral displays and rewards, either because lower-latitude plants require greater pollination rates to achieve an equivalent level of fruit set to higher-latitude plants, or because greater pollination competition at lower latitudes requires more attractive displays to achieve an equivalent pollination rate. To compare floral displays and reward, in 2016 we planted a cohort of 81 plants in a greenhouse common garden at Michigan State University. The experiment consisted of three populations each from tropical, subtropical, and temperate regions (Table 1), with three maternal lines per population (seeds collected from one individual in the field) and three individuals per maternal line. For one population (Finca Bellavista), one maternal line had two plants and there was a fourth line with one plant. These plants were grown simultaneously and with the same methods as the common garden in Baskett and Schemske (2018), but it is not the same cohort.

Floral display size was quantified by counting open flowers per inflorescence (with tepals, without swollen pistil) on three inflorescences per plant (one plant was missing measurements from two inflorescences), which may or may not have been measured on the same day. Measurements were made between late July and November (median August 23); tropical plants flowered later. Flower counts were analyzed using a mixed-effects model with the fixed effect of region and the random effect of individual nested in maternal line nested in population. Variance was estimated separately for each population.

Nectar was measured on a combined 10 flowers per plant (from ~3 inflorescences) using a 1-µL microcapillary tube. To access nectaries, the pistil was removed with forceps. Measurements were made between 09:30 and 12:00 on up to three separate mornings per plant between late July and November (median September 20). We were able to measure nectar for 80 of the 81 plants. For analysis, we averaged nectar volume per flower across up to three replicate measurements per individual, because only 60% of individuals had three replicates. Nectar volume was analyzed using a mixed model with a fixed effect of region and a random effect of maternal line nested in population.

To estimate pollination rates, we used videos of insect visitation to flowers over multiple seasons in native habitats. We quantified field visitation in three tropical populations of *P. rivinoides* and three north-temperate populations of *P. americana* (Table 1, Fig. 1a), as we were logistically constrained to conduct repeated monitoring in only two regions. Tropical populations are the same as those used for the common garden, but two of the temperate populations are not (Fig. 1a). Visits were recorded with GoPro cameras (HERO3+ and HERO4; GoPro, San Mateo, California, USA), positioned 30–50 cm away from inflorescences (examples in Fig. 1; Video S1). In the tropics, videos were recorded during three observation blocks, spaced six weeks apart: dry season, dry-wet transition, and wet season (April 11–July 24, 2016). In the temperate region, we recorded in three observation blocks, spaced three to four weeks apart during the flowering season (5 July–24 August 2017). During each observation block, two to four cameras were deployed for 1–3 d in each population. Cameras were rotated among flowering individuals each day, and in each observation block the median number of videos per plant was one, although some plants were observed up to four times (never the same inflorescence on the same day). The unit of replication is a video of a particular inflorescence on a particular day. Video duration ranged from 20 to 160 min (median = 62), and we recorded between 7:30 and 16:30. Overall, we recorded 177 usable hours of video from 97 plants, resulting in 104 estimates of visitation rate (115 h) in tropical populations and 62 estimates (62 h) in temperate populations. For identification purposes, 23 *P. americana* visitors were collected in southeast Michigan and 32 *P. rivinoides* visitors were collected in Costa Rica. Pollen loads were examined by T. Wood and compared to a reference library.

To estimate visitation rates, we recorded the number of flowers visited in each video (contact with anthers or stigma). All visitors were insects. We excluded ants and lepidopteran larvae, which were judged not to be legitimate pollinators based on their morphology and foraging behavior. Rainy or windy videos were excluded because these conditions reduce insect activity and recording visibility. In the field, we counted total number of open flowers on each inflorescence to use display as a covariate and test whether per-flower visitation increases nonlinearly with display size. Flowers were counted before and after each video (in case any opened during filming), and the mean of these two counts was used for analysis. We used the response variable of visits per flower per hour (total visits were divided by the number of open flowers and video duration). The response was $\log_e(x + 1)$-transformed to meet assumptions of normally distributed residuals and analyzed using a mixed model with fixed effects of region, open flowers, and their interaction. The effect of display size tests whether a larger display increases visits per flower, not per inflorescence, as the response is standardized by flower number. The interaction effect tests whether such an effect of display size differs by region. The model included the random effect of individual nested within population, and variance was estimated separately for each population. We also asked whether there was seasonal variation in floral visitation; regions were analyzed separately because their seasons are not comparable. Floral visitation was analyzed as a function of season with random effects of individual nested in population and separate estimates of variance for each population.

We evaluated self-pollination ability in the absence of pollinators (autogamy rate) for tropical, subtropical,

and temperate plants in another greenhouse common garden at Michigan State University (Table 1). This was a separate cohort from the floral traits study, but they were grown at the same time with the same methods. Seeds were germinated in summer 2016 and autogamy was estimated from January to August 2017. For each population, we attempted to grow three plants from each of three maternal lines, resulting in a total of 74 plants (22–27 per region). We sampled up to three mature infructescences per individual and counted the number of total flowers and filled fruits (>50% swollen). Autogamy rate was calculated as the ratio of filled fruits vs. flowers. Autogamy rate (individual means) was analyzed with a mixed-effects model as a function of region, with maternal line nested in population as a random effect. Variance was estimated separately for each population.

Plant–herbivore interactions

To compare herbivore pressure from chewing insects between tropical and temperate *Phytolacca*, we surveyed herbivory rates in tropical and temperate regions (Table 1). Young and mature leaves were marked and re-measured in order to capture complete consumption and standardize over time to compare to other studies (Coley 1983, Coley and Barone 1996, Anstett et al. 2016, Baskett and Schemske 2018). Herbivory rate was measured over three intervals in the tropics between 4 March and 23 July 2016; each interval was 38–47 d (median = 43), and the three intervals covered the end of the dry season, the dry-wet transition, and the beginning of the wet season (Appendix S1: Table S2). Herbivory rate was measured over two intervals in the temperate zone, due to its shorter growing season, between 5 July and 29 September 2017. Each interval was 40–43 d (median = 41), and the two intervals covered mid and late summer, after the rapid early summer growth (Appendix S1: Table S2).

Herbivory rate (chewing damage) was measured following protocols of Baskett and Schemske (2018), a separate study of herbivory rates along a latitudinal gradient in *P. americana*. In short, at the start of each interval, five mature and five young leaves were marked per plant for 20–30 plants per population, using colored bird bands and permanent marker. Initial percent damage was measured on mature leaves ([consumed area/total leaf area] × 100) using a transparent plastic grid with 6.35 × 6.35 mm squares. Young leaves were marked when buds were bursting. All young leaves began with zero initial herbivory. An average of 203 and 300 leaves per population per season were marked in tropical and temperate regions, respectively, for a total of 3,625 marked leaves. Final percent consumption was measured approximately six weeks later, after most young leaves had expanded. Due to plant mortality, at the end of each interval we were able to measure on average 18 plants in tropical and 29 plants in temperate regions per population per season.

To test whether herbivore pressure varies with region, we analyzed mean per-plant herbivory using a mixed model with fixed effects of region, leaf age, and their interaction, and random effects of individual nested in population, due to repeated measures over multiple seasons. Variance was estimated separately for each leaf age in each population. For clarity, we present results for final minus initial consumption, expressed as a percentage, because the interval over which we measured was similar for all populations (39–44 d; Appendix S1: Table S2). Analyzing herbivory as a daily rate does not qualitatively change results. To check for seasonal variation, herbivory was analyzed separately for each region as a function of season, leaf age, and their interaction; individual nested in population was a random effect.

Concurrent with herbivory surveys, we collected lepidopteran larvae in each population to estimate herbivore abundance. There were four censuses in each region: at the first leaf-marking and last leaf-measurement, and two in between (three to six week intervals). At each census, we searched the top and bottom surfaces of 3,000 leaves of all sizes per population, divided among the individuals surveyed for herbivory. Larvae were counted as morphospecies, and we attempted to rear representative specimens to adulthood on a *Phytolacca* diet for identification.

Herbivore abundance was $\log_e(x + 1)$-transformed for analysis to meet assumptions of normally distributed residuals. We removed singletons (morphospecies that were only collected once out of 36,000 leaves checked in each region), assuming they are not important consumers, which did not qualitatively change results. Total abundance from 3,000 leaves in each population at each census was analyzed using a mixed-effects model with census as a random effect to account for repeated measures of populations. Variance was estimated separately for each region. We asked whether herbivory correlates with herbivore abundance with a Pearson correlation of mean herbivory and mean herbivore $\log_e$(abundance) per site. Abundance was measured without regard to leaf age, so leaf age was not included in analysis.

To compare latitudinal patterns in evolution of plant defense, we performed a bioassay using performance of *Spodoptera exigua* larvae (Lepidoptera, Noctuidae) as a metric of strength of plant defense. We chose this species because it has a highly generalized diet, but does not consume *Phytolacca* in nature, so there is no risk of local adaptation to certain populations, particularly since there is no generalist species that spans the entire latitudinal gradient studied. In our experience, *S. exigua* tolerates a *Phytolacca* diet better than other commercially available caterpillars. We used palatability data from Baskett and Schemske (2018) for subtropical and temperate *P. americana* and here include new data on three tropical populations of *P. rivinoides* from the same experiment. Detailed experimental methods are described in Baskett and Schemske (2018). In brief, neonate larvae were fed a no-choice diet of young or mature

leaves of *P. americana* or *P. rivinoides* from a greenhouse common garden. Larvae were reared in cups (the experimental unit); each cup received leaves from multiple plants in one maternal line. For the mature-leaf treatment, cups began with five individuals, the experiment ran for 8 d, and there were four replicate cups per maternal line. Young-leaf material was more limited, so cups began with three individuals and ran for 9 d, with three replicate cups per maternal line. Larvae were not food limited, and they began the experiment as tiny neonates, so we assumed there were no effects of starting larval density despite its confounding with leaf age treatment. In addition, density changed throughout the experiment due to larval mortality. We measured total larval biomass of survivors per cup at the end, counting non-survivors as zero biomass to integrate survival and growth in one response, which we call "palatability" to distinguish from growth alone.

For analysis of palatability, larval biomass per cup was $\log_e(x + 1)$-transformed to linearize exponential growth. After transformation, biomass was standardized by the number of starting caterpillars and the duration of the trial to compare leaf age treatments (5 individuals × 8 d for mature, 3 individuals × 9 d for young leaves). We analyzed palatability as a function of region, leaf age, and their interaction, with a random effect of maternal line nested in population. Variance was estimated separately for each leaf age in each population. Additionally, survival and growth were analyzed separately to decompose palatability into its component parts. Unlike all other mixed models here (see *Statistical analyses common across studies*), proportion surviving per cup (specifying initial caterpillar count) was analyzed with the glmer function in the lme4 package in R, using the binomial family. Fixed effects were region, age, and their interaction, and we included a random effect of maternal line nested in population. It is not possible to estimate denominator degrees of freedom with this function; we obtained *P* values for fixed effects with likelihood ratio tests. Growth is mean biomass of surviving caterpillars per cup, $\log_e(x + 1)$-transformed to linearize exponential growth. Cups with no survivors were excluded (41 cups; note that they and all other dead caterpillars are included in the palatability analysis above as zero biomass). Growth was analyzed as a function of region, age, and their interaction, with random effects of maternal line nested in population. Variance was estimated separately for each population.

RESULTS

Plant–pollinator interactions

The tropical *Phytolacca rivinoides* had a 2.5-fold larger floral display than *P. americana* in a common garden, as measured by open flowers per inflorescence (Fig. 2). There was no difference in display size between temperate and subtropical *P. americana* (Fig. 2). Nectar

volume was similar across regions, though subtropical plants produced marginally more microliter nectar per flower (least-square means ± SE, tropical 0.049 ± 0.004, subtropical 0.065 ± 0.004, temperate 0.049 ± 0.004; $F_{2,6} = 4.73$, $P = 0.0586$).

Measured from field videos, tropical *P. rivinoides* flowers were visited 2.8 times per hour on average, a 13-fold higher rate than temperate *P. americana* flowers (least-square mean of log-transformed visitation was 8-fold higher; $F_{1,4} = 35.72$, $P = 0.0039$, Fig. 3). There was no significant effect of floral display size (number of open flowers) on visits per flower ($F_{1,67} = 3.25$, $P = 0.0761$); that is, a flower in a larger display receives the same number of visits as a flower in a smaller display after accounting for effects of region. Nor was there a significant display–region interaction ($F_{1,67} = 1.72$, $P = 0.1941$). During the last temperate observation block, one population was no longer flowering and another had only one inflorescence, but there was no significant seasonal variation in floral visitation rates in either the tropics ($F_{2,59} = 0.88$, $P = 0.4212$) or temperate zone ($F_{2,6} = 4.51$, $P = 0.0638$).

Visitors to *P. americana* and *P. rivinoides* included small bees (especially *Lasioglossum*, but also *Augochlora*, *Augochlorella*, *Ceratina*, and *Trigona*), Vespidae (*Parancistrocerus*, *Stenodynerus*), Diptera (Syrphidae, Tephritidae), and Coleoptera (Curculionidae, Chrysomelidae; Appendix S1: Tables S3, S4). Out of the visitors we collected for identification purposes, bees were the only visitors with *Phytolacca* pollen on their bodies (5 of 10 temperate bees, 13 of 27 tropical bees; Appendix S1:
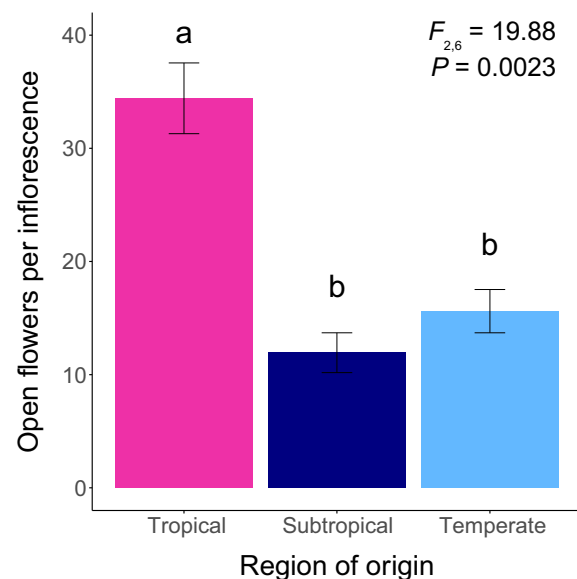

FIG. 2. Floral display size in a common greenhouse environment of three populations per region for *Phytolacca rivinoides* (pink) and *P. americana* (dark and light blue). Bars are least-square means ± SE. Bars with different letters are significantly different.
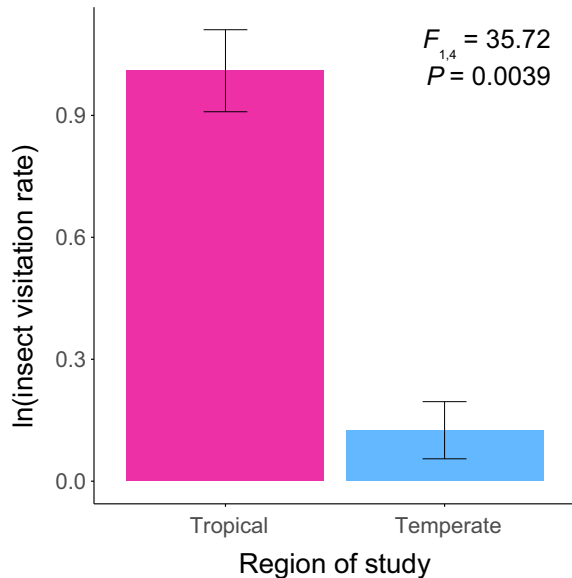
FIG. 3. Insect visitation rate (visitors per flower per hour) in situ for three populations each of tropical *Phytolacca rivinoides* (pink) vs. temperate *P. americana* (blue). Bars are least-square means ± SE.
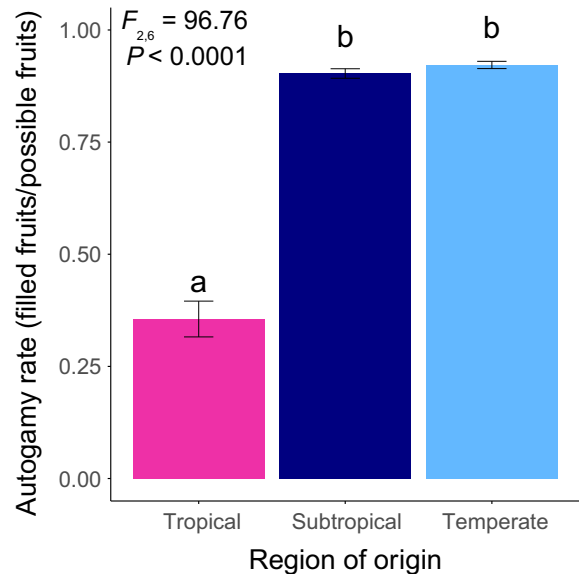

FIG. 4. Autogamy rate (filled/possible fruits) for *Phytolacca rivinoides* (pink) and *P. americana* (dark and light blue) in a greenhouse. Each region was represented by three populations. Bars are least-square means ± SE, and significant differences between regions are indicated with different letters.

Table S3). The low resolution of pollinator videos did not permit us to match visitors to specimens. Instead, when possible we assigned morphospecies to order, and within Hymenoptera, to bees (Apoidea). Bees constituted 49% of *P. americana* visitors and 88% of *P. rivinoides* visitors (Appendix S1: Table S4).

Phytolacca americana populations from both subtropical and temperate regions had a high autogamy rate in the greenhouse, with 91% of possible fruit set. This was 2.6-fold higher than the 36% autogamy rate of *P. rivinoides* (Fig. 4). In April, we observed a small number of wasps (unidentified Vespidae) in the greenhouse, suggesting that pollinator exclusion was not completely effective. Thus, for both species, rates may be overestimated because of incomplete pollinator exclusion; estimated autogamy was 84% in preliminary studies of the same populations of *P. americana* with complete exclusion, and usually when we grow *P. rivinoides* in the greenhouse, we observe almost no fruit set (C. Baskett, *personal observation*).

Plant–herbivore interactions

Herbivory in the field depended on both region and leaf age (Fig. 5a; region $F_{1,4} = 11.88$, $P = 0.0261$; leaf age $F_{1, 507} = 39.76$, $P < 0.0001$; interaction $F_{1, 507} = 23.20$, $P < 0.0001$). Young tropical leaves were consumed at 4.8 times the rate of young temperate leaves. An even larger effect was observed for mature leaves, which were consumed at a 11-fold higher rate in the tropics vs. temperate populations, where consumption was essentially zero. Within both regions, herbivory was greater on

young than mature leaves: a 2.3-fold difference in the tropics and a 5.4-fold difference in the temperate region. Tropical populations experienced a 79% increase in herbivory from dry to wet season on young leaves, but no seasonal herbivory changes for mature leaves (Fig. 5b; season $F_{2, 244} = 0.28$, $P = 0.7559$; leaf age $F_{1, 244} = 6.56$, $P = 0.0110$; interaction $F_{2, 244} = 3.84$, $P = 0.0229$). There was no seasonal effect on herbivory in temperate populations (season $F_{1, 257} = 3.54$, $P = 0.0611$; leaf age $F_{1, 257} = 17.80$, $P < 0.0001$; interaction $F_{1, 257} = 0.44$, $P = 0.5056$). Daily herbivory rates for each population at each interval are provided in Appendix S1: Table S2.

Lepidopteran larval abundance (per 3,000 leaves) was 13.8 in tropical populations, which is 41-fold higher than temperate populations (least-square mean of log-transformed abundance was 10-fold higher; $F_{1,6} = 27.43$, $P = 0.0019$, Fig. 6). Seven morphospecies of 165 individuals were observed in the tropics. The most common (61% of observed larvae) was a Crambidae species found consuming and sheltering in young leaves (possibly *Maracayia*), and the second-most common (19%) was *Disclisioprocta stellata* Guenée (Geometridae). Two species, each at 6–7% abundance, never survived to adulthood; one is likely either Crambidae or Tortricidae, and the other is unknown. Two Tortricid moths and a Noctuid (possibly *Tiracola grandirena* Herrich-Schäffer and/or *Spodoptera eridania* Stoll) were each found at 1–4% relative abundance. The four families collected on *P. rivinoides* were the same as those observed consuming *P. americana* in the eastern United States, at roughly similar relative abundances (Baskett and Schemske 2018). In the temperate zone in the present study, we
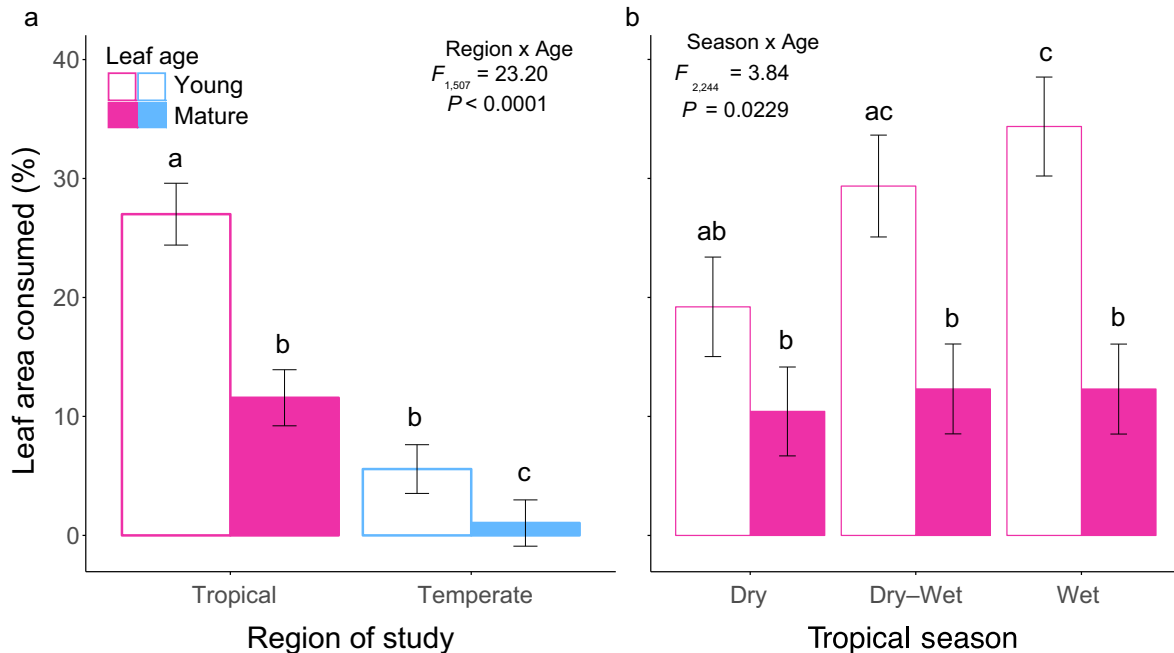
FIG. 5. (a) Percent leaf area consumed of young and mature leaves in three populations each of tropical *Phytolacca rivinoides* (pink) and temperate *P. americana* (blue) averaged over multiple seasons. Herbivory was surveyed by marking and remeasuring leaves ~42 d later over three intervals (b) in the tropics and two in the temperate zone (data not shown because there was no significant effect of season). Bars are least-square means ± SE, and those that share the same letter within a plot are not significantly different according to Tukey's HSD test.

only found four individuals of two morphospecies after surveying 36,000 total leaves in three populations. None survived to adulthood. This is a lower abundance than in 2015, when we observed seven individuals of *Argyrotaenia velutina* upon surveying 6,000 leaves in one of the temperate populations used in the present study (Baskett and Schemske 2018). Even if the 2015 north-temperate abundance is extrapolated, tropical populations still had 4-fold the number of larvae. Lepidopteran $\log_e$(abundance) is strongly positively correlated with mean herbivory in each population (Pearson's $r = 0.98$, $t_4 = 10.08$, $P = 0.0005$).

Palatability, assayed by *S. exigua* caterpillars, depended on the interaction of region and leaf age (Fig. 7a; region $F_{2,6} = 27.00$, $P = 0.0010$; leaf age $F_{1, 239} = 262.02$, $P < 0.0001$; interaction $F_{2, 239} = 13.15$, $P < 0.0001$). Recall that palatability integrates survival and growth by counting non-survivors as zero biomass. The effect of leaf age within region was consistent: mature leaves were always more palatable than young leaves, with the largest difference in the tropics. Within the young leaves, the tropical diet caused the poorest larval performance, the temperate diet had the highest palatability, and subtropical was intermediate. Caterpillars consuming a tropical young-leaf diet attained 17% of the $\log_e$(biomass) compared to the subtropical young-leaf diet. In turn, larvae eating subtropical young leaves had only 9% of the $\log_e$(biomass) of those eating temperate young leaves. In contrast, regional patterns for
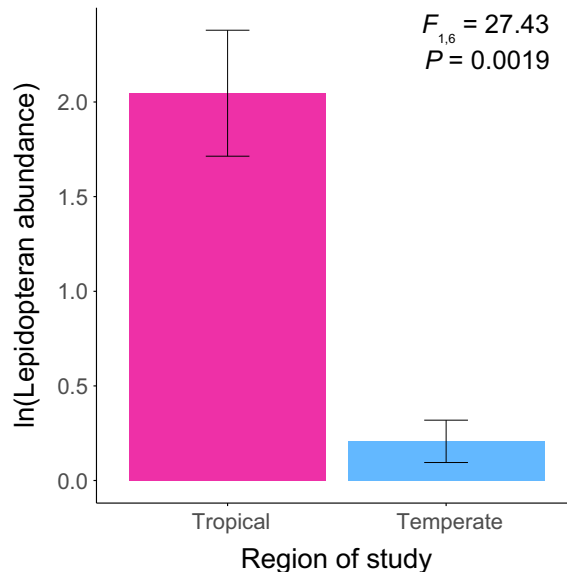

FIG. 6. Abundance of lepidopteran larvae in three populations each of *Phytolacca rivinoides* (pink) and *P. americana* (blue). Four censuses of 3,000 leaves were conducted in each population. Bars are least-square means ± SE.

mature leaves differed from young leaves. There was no significant difference between palatability of tropical and temperate mature leaves. The subtropical mature-leaf
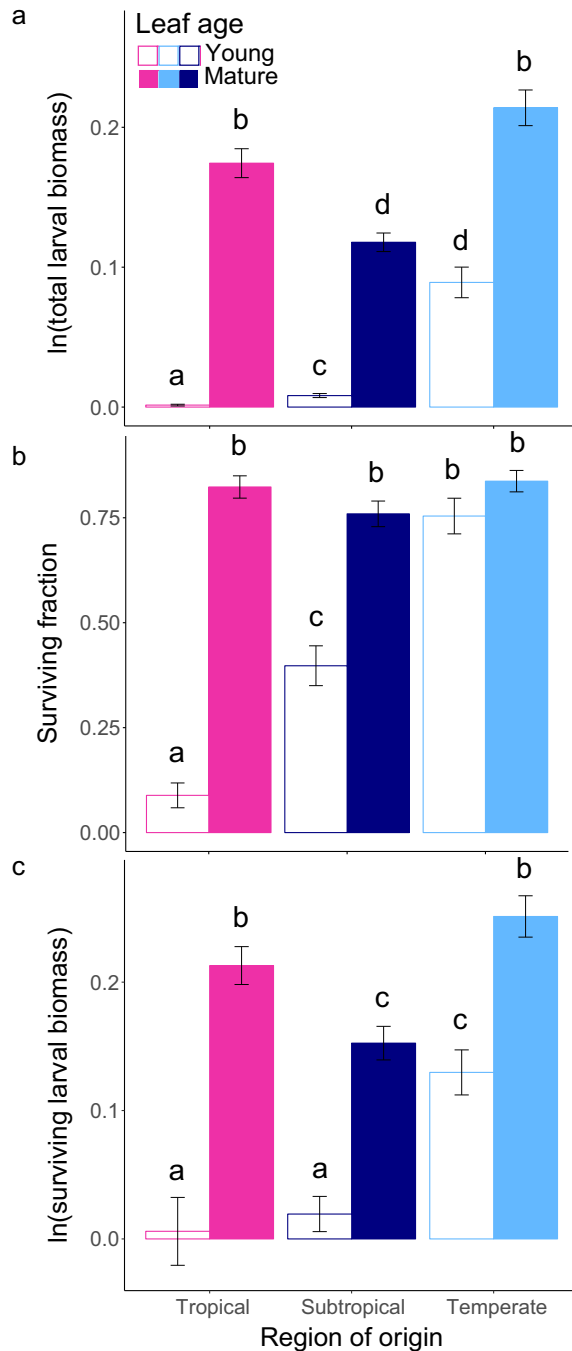
FIG. 7. (a) Palatability and its components of (b) survival and (c) growth as assayed by *Spodoptera exigua* caterpillars reared on no-choice *Phytolacca* diets. Diets were young or mature leaves of greenhouse-grown *Phytolacca rivinoides* (pink) and *P. americana* (dark and light blue). Palatability integrates survival and growth by counting dead larvae as zero biomass. Three plant populations per region were assayed. Bars are least-square means ± SE; those that share the same letter within a plot are not significantly different, according to Tukey's HSD (there was a significant interaction of region and leaf age for each response; see *Results*). Data for subtropical and temperate populations were previously reported in Baskett and Schemske (2018). Biomass was measured as milligrams.

diet reduced larval $\log_e$(biomass) to 61% of that on a tropical or temperate mature-leaf diet.

Patterns of palatability were mostly driven by caterpillar growth, though survival also played a role for the two diets with lowest palatability (tropical and subtropical young leaves). Survival rate depended on the interaction of region and leaf age (Fig. 7b; region $F_2 = 9.23$, $P = 0.0003$; leaf age $F_1 = 99.23$, $P < 0.0001$; interaction $F_2 = 25.18$, $P < 0.0001$; denominator df could not be calculated). Nearly 80% of larvae survived in most treatments. However, larvae consuming tropical young leaves had the lowest survival, only 9%. Compared to the tropical young leaves, survival was 4-fold higher in the subtropical young-leaf treatment and 9-fold higher in the four other treatments. Growth of surviving caterpillars also depended on the interaction of region and leaf age (Fig. 7c; region $F_{2,6} = 12.0$, $P = 0.0080$; leaf age $F_{1, 198} = 280.4$, $P < 0.0001$; interaction $F_{2, 198} = 4.6$, $P = 0.0108$). Comparisons of growth among treatments showed very similar results to palatability, except that there was no significant difference in size of caterpillars that survived on a tropical and subtropical diet; their difference in palatability was driven by survival (Fig. 7). Surviving caterpillars consuming tropical or subtropical young leaves attained 10% of the $\log_e$(biomass) of those consuming temperate young leaves. Surviving caterpillars consuming subtropical mature leaves obtained 66% of the $\log_e$(biomass) of caterpillars consuming tropical or temperate mature leaves.

DISCUSSION

A latitudinal trend in the strength of species interactions has been long hypothesized to drive biodiversity patterns, since Dobzhansky speculated that "the challenges of tropical environments stem chiefly from the intricate mutual relationships among the inhabitants" (1950). In plants, pollination and herbivory are important and well-studied biotic interactions generally, and there may be more latitudinal comparisons of these biotic interactions than any others (Schemske et al. 2009). Despite the relatively high attention paid to these interactions, there are still significant gaps in the approaches taken thus far, which could explain a lack of consensus regarding support for the biotic interactions hypothesis (Anstett et al. 2016, Moles and Ollerton 2016). For example, latitudinal patterns in pollination have only been evaluated using community-level approaches, and few studies have provided in-depth comparisons of the importance of herbivory across a temperate-tropical range. Here, we quantified multiple proxies of interaction importance across a broad latitudinal range using the temperate herb *Phytolacca americana* and its tropical congener *P. rivinoides*. Consistent with the biotic interactions hypothesis, nearly all measures of pollination and herbivory showed evidence of greater importance of biotic interactions in the tropical *P. rivinoides* than north-temperate *P. americana:* floral display size,

pollination, autogamy, herbivory rate on young and mature leaves, herbivore abundance, and palatability of young leaves (see Table 2 for summary and respective figure references). Within *P. americana*, there is evidence for greater herbivore pressure and defense at lower latitudes (Baskett and Schemske 2018). However, results for pollination within *P. americana* did not support the biotic interactions hypothesis, as temperate and subtropical populations did not differ for floral display, nectar, or autogamy. In contrast, agreement among several metrics of mutualistic and antagonistic interactions bolsters our conclusion that tropical *P. rivinoides* experiences greater contemporary interaction strength and has evolved traits consistent with a history of stronger interactions compared to its temperate congener.

We hypothesized that importance of herbivory and pollination would be greatest in the tropics, intermediate in the subtropics, and least in the temperate regions studied. Indeed, at the largest scale of our study, comparing tropical *P. rivinoides* (10° N) to temperate *P. americana* (41° N), all but two metrics of the importance of herbivory and pollination supported the biotic interactions hypothesis (Table 2; nectar volume and mature-leaf palatability were equivalent between the two regions). However, the data from subtropical *P. americana* (~28° N), although less complete (e.g., no pollination in the field), demonstrated a variety of geographic patterns (Table 2), in contrast to our hypothesis that interaction importance would always be intermediate those of the other two regions. Subtropical young-leaf palatability was indeed intermediate, while mature subtropical leaves were better defended compared to both temperate and tropical regions, and autogamy and floral display were similar between subtropical and temperate populations (Table 2). A lack of the hypothesized latitudinal pattern in a trait within a species could be due to strong, consistent selection pressure across the species' range swamping any potential effects of latitude on selection, and/or due to high gene flow among populations countering small geographic differences in

selection. A lack of the hypothesized latitudinal pattern between species could be mediated by a myriad of inter-specific differences that mediate fitness impacts of biotic interactions, e.g., in life history, phenology, or herbivory tolerance. More studies spanning tropical and subtropical regions are needed to clarify latitudinal patterns at the edge of the tropics and identify putative mechanisms underlying geographic variation in interactions. We suggest that single-species studies are useful for this purpose, in that they provide stronger experimental control of history and ecology than is possible with congeners. To our knowledge, there are no single-species, fine-scale, latitudinal gradients that have been sampled in the subtropical-tropical range for herbivory. There is one example at a course scale: in a comparison of herbivory in three sites in the mangrove *Rhizophora mangle*, higher folivory was observed in Florida than Belize (27° vs. 17° N), and Panama (9° N) did not significantly differ from either site (Feller et al. 2013).

The variation in results for the subtropical region suggests that latitudinal patterns in interaction importance are not simply linear, although we lack intermediate sites that would clarify the shape. With three sites, we can only speculate that harsh winters impose constraints on *P. americana* or interacting insects that limit biotic interaction importance in the temperate zone compared to the tropics, and that subtropical winters with less frequent freezing affect biotic interactions in idiosyncratic ways. To give a rough sense of how the extratropical winters compare, average winter minimum temperature is 10°C in Florida and −9°C in Michigan (Arguez et al. 2010). Linear latitudinal patterns seem to be an implicit assumption in tests of the biotic interactions hypothesis (including this one), but perhaps this assumption is worth re-visiting in light of evidence of nonlinear patterns. For example, the proportion of mycorrhizal plant species decreases with latitude more rapidly outside than inside the tropics (Delavaux et al. 2019). Our previous study also found evidence of nonlinearity in species interactions: when we compared herbivory and defense

TABLE 2. Summary of predictions from the biotic interactions hypothesis, results of this study, and whether results provide support (support indicated by daggers) for predictions for tropical *Phytolacca rivinoides* versus temperate and subtropical *P. americana*

Interaction or trait	Prediction: low-latitude plants have...	Results					Figure
Floral display	larger display	tropical	>[†]	subtropical	=	temperate	2
Floral reward	more nectar	tropical	=	subtropical	=	temperate	n/a
Floral visitation rate	higher visitation rate	tropical		>[†]		temperate	3
Self-pollination (autogamy)	lower autogamy rate	tropical	<[†]	subtropical	=	temperate	4
Herbivory rate: young leaves	higher herbivory rate	tropical		>[†]		temperate	5a
Herbivory rate: mature leaves	higher herbivory rate	tropical		>[†]		temperate	5a
Herbivore abundance	higher herbivore abundance	tropical		>[†]		temperate	6
Palatability: young leaves	less palatable leaves	tropical	<[†]	subtropical	<[†]	temperate	7a
Palatability: mature leaves	less palatable leaves	tropical	>	subtropical	<[†]	temperate	7a

Notes: Direction of significant differences is indicated by < or >, while = indicates no significant difference. Results for subtropical *P. americana* populations are not shown for some metrics because they were not measured in all studies (Table 1).

of 10 *P. americana* populations in order to describe the shape of latitudinal patterns, we found a threshold in herbivory at 35° N and a threshold in palatability at 30° N (Baskett and Schemske 2018). Such thresholds in biotic interactions could be caused by discreet adaptations to continuous climatic gradients (e.g., number of life cycles insects can complete depends on growing season length; Scriber et al. 2014). It is unclear whether nonlinear latitudinal patterns in biotic interactions are unusual, as studies typically do not look for them (Moles et al. 2011*a*). But such patterns point out that latitude is a problematic predictor variable because it is confounded with several climatic factors and with biogeographic history, and does not include significant climatic variation due to elevation. Thus, there is a need to refine tests of the biotic interactions hypothesis by quantifying climate instead of using latitude as a proxy, in order to generate and test hypotheses for causal mechanisms driving geographic variation in biotic interactions (Schemske et al. 2009, Pearse and Hipp 2012, Anstett et al. 2016). Clearer relationships with climate could help clarify not only causes of variation in biotic interaction importance, but also its consequences for species richness, which has been found in some cases to be nonlinearly related with abiotic (Currie 1991, Tittensor et al. 2010) and biotic (LaManna et al. 2017) components of the environment. Such research is challenging because studies would need larger sample sizes to test relationships with multiple climatic variables compared to simply testing relationships with latitude. However, an exciting potential benefit of focusing on climate is integration of the biotic interactions hypothesis with related frameworks. For example, the plant defense literature has had some success relating defense to climate by testing the biotic interactions hypothesis, the "resource availability hypothesis," or both (Pearse and Hipp 2012, Moreira et al. 2015, 2018, Hahn and Maron 2016, Hahn et al. 2019).

Most latitudinal comparisons of plant–pollinator interactions thus far have been at the community level, so it is difficult to contextualize our contrasting results for the interspecific and intraspecific comparisons. The prevalence of animal pollination increases toward the tropics (Regal 1982, Rech et al. 2016), as does reliance of vertebrate-pollinated plants on their pollinators (Ratto et al. 2018). For animal-pollinated plants, outcrossing rate increases toward the equator, but this pattern is driven by latitudinal variation in life histories because there are more herbaceous taxa at higher latitudes and these tend to have higher selfing rates (Lloyd 1980, Moeller et al. 2017). Pollen limitation is greatest in species-rich communities, which is thought to be a signature of greater competition for pollinators (Vamosi et al. 2006). Interaction specialization has also sometimes been considered an index of interaction importance, but latitudinal patterns of specialization in plant–pollinator interactions remain unresolved (Olesen and Jordano 2002, Kay and Schemske 2004, Dalsgaard et al. 2011,

Ollerton 2012, Pauw and Stanway 2015). We only know of one other study of latitudinal patterns in pollination importance within a species, the herb *Halenia elliptica* (Gentianaceae). Yang et al. (2018) found that outcrossing rates negatively correlate with latitude over 11° in the temperate zone, consistent with the biotic interactions hypothesis. Additionally, *Arabis alpina* (Brassicaceae) shifts from self-incompatible in southern Europe to highly selfing in northern Europe, although the study was not framed as a latitudinal analysis (Laenen et al. 2018). Here, we found that the tropical *P. rivinoides* invested in larger floral displays, was visited at higher rates by insects in nature, and had poorer self-pollination ability than temperate *P. americana*. Taken together, these results suggest that *P. rivinoides* requires greater pollination rates than *P. americana* to achieve an equivalent level of fruit set. There is no effect of display size on visitation per flower after accounting for region, so further research would be needed to determine whether the interspecific variation in display size causes the interspecific differences in visitation rate. As with any interspecific comparison, these results must be interpreted with caution because they may be driven by differences other than latitude. However, the only major known difference in the species' ecology is life history: *P. americana* is iteroparous, while *P. rivinoides* is semelparous and shorter-lived. But benefits of selfing are predicted to decrease with species' life span (Morgan et al. 1997), which would predict higher selfing for *P. rivinoides*. The pronounced interspecific differences contrast with our findings of no evidence for regional differentiation in plant–pollinator interactions of *P. americana* populations at the northern and southern edge of the range. Perhaps this lack of differentiation is explained by the fact that *P. americana* was capable of high autogamy rates in both regions, consistent with a weedy, gap-specialized ecology (Sauer 1952, Lloyd 1980, Pannell et al. 2015). Future studies should clarify the importance of pollinators to subtropical populations by measuring in situ visitation, pollen limitation, and outcrossing rates between regions. We also caution that measuring floral traits in a common environment is challenging for such a large plant that is likely space and resource limited in a greenhouse, especially because higher-latitude plants flower earlier. Here we have presented the best available data out of multiple attempts at common garden measurements of floral traits. While the display size differences here were consistent between our studies, in two smaller studies we found that tropical and/or subtropical plants produced significantly more nectar than temperate plants (Baskett 2018; C. Baskett, *unpublished data*).

In contrast to pollination, latitudinal patterns in herbivory and plant defense have been studied extensively using both community- and species-level approaches. However, evidence for the biotic interactions hypothesis is mixed (Moles et al. 2011*a*), possibly due to inconsistencies and biases in commonly used methods (Anstett et al. 2016), such as a lack of damage estimates on young

leaves (but see Baskett and Schemske 2018). We found evidence of stronger contemporary herbivore pressure (herbivory rate and herbivore abundance) in tropical vs. temperate plants, consistent with the biotic interactions hypothesis. Consistent with previous comparisons of herbivory on leaves of different ages, we found that young leaves experienced significantly greater herbivory (Coley 1983, Lowman 1984, Filip et al. 1995, Baskett and Schemske 2018), likely due to their reduced toughness and greater nitrogen content compared to mature leaves (Baskett 2018). In addition, we confirmed previous findings that wet-season herbivory is greater than dry-season herbivory in the tropics (Coley 1983, Aide 1993, Coley and Barone 1996). In fact, herbivory on *P. rivinoides* was remarkably similar to that measured across 20 other gap-specialized plants in 1979 in Panama by Coley (1983), except that we saw higher mature-leaf herbivory in the dry season (Table 3). The present study did not quantify field herbivory for subtropical *P. americana*, but previously we observed approximately twofold higher herbivory rates on both young and mature leaves of *P. americana* in late summer in the southern United States (Florida and Georgia) compared to wet-season Costa Rican *P. rivinoides* in the present study (Baskett and Schemske 2018). Further work is needed to understand whether this observation is inconsistent with the biotic interactions hypothesis, as we lack sufficient data across entire growing seasons to estimate annual herbivory rates in either region, and we lack data on fitness effects of herbivory and herbivory tolerance in *Phytolacca* (Lehndal and Ågren 2015a, Anstett et al. 2016).

Plant defense, a metric of how plants have evolved in response to their history of herbivore pressure, provides important evolutionary context for interpreting latitudinal patterns in contemporary herbivory (Salgado and Pennings 2005, Woods et al. 2012, Kim 2014, Anstett et al. 2015, Lehndal and Ågren 2015b, Moreira et al. 2018, Hahn et al. 2019). This is because three possible patterns of herbivory and defense would support the biotic interactions hypothesis: stronger tropical defense required to reduce herbivory to similar levels; similar defense but greater damage in the tropics; or both greater herbivore pressure and greater defense in the tropics. Our results are consistent with the latter, and

TABLE 3. Mean proportion of leaf area consumed per day by leaf age in dry and wet seasons in Central America.

Season	Leaf age	Gap spp. 1979	*P. riv.* 2016
Dry	young	0.46	0.44
Dry	mature	0.13	0.29
Wet	young	0.89	0.85
Wet	mature	0.28	0.27

Notes: Gap spp. means of 20 gap specialists, not including *P. rivinoides*, measured in Panama by Coley in 1979, from Table 1 in Coley (1983). *P. riv.*, *Phytolacca rivinoides* in three populations in Costa Rica in 2016 (this study).

suggest that *Phytolacca* herbivores causing damage in the field are not deterred by the defensive traits that negatively impact the naïve generalist in the bioassay experiment. We found that mature leaves were more palatable to the generalist *S. exigua* than young leaves in all populations, similar to a study of *Verbascum* (Alba et al. 2014), likely due to chemical defenses in young leaves that are unpalatable to generalists (Baskett 2018). We found that young *P. rivinoides* leaves were highly unpalatable, more than any other diet. Despite uncertainty about how annual herbivory rates compare between tropical and subtropical regions, the difference in palatability between these two regions is consistent with the hypothesis that herbivore pressure on young leaves in *Phytolacca* has indeed been strong in the tropics. Compared to temperate *P. americana*, mature *P. rivinoides* leaves were equally palatable but consumed at higher rates in the field, which is consistent with greater herbivore pressure in the tropics. But curiously, compared to subtropical *P. americana*, mature *P. rivinoides* leaves were more palatable. It is unclear why mature leaves show different latitudinal patterns than young leaves; performing palatability trials with other species could clarify the generality of plant defense patterns for both leaf ages. Furthermore, latitudinal patterns in both herbivory and defense must be mediated by the fitness effects of herbivory, which could be affected by differences in seasonality, in life history (*P. americana* is longer lived), or in herbivory tolerance, which we did not measure (Stowe et al. 2000, Anstett et al. 2016).

To our knowledge, previous work on latitudinal patterns in biotic interactions has not integrated studies of antagonistic and mutualistic interactions in the same system. The biotic interactions hypothesis does not make distinct predictions for different types of interactions. Mutualistic and antagonistic coevolution may have different outcomes for diversification, but this idea is just beginning to be explored (Hembry et al. 2014, Baskett and Schemske 2015); future refinements of the biotic interactions hypothesis may need to distinguish among interaction types. We found similar patterns for herbivory and pollination importance for the temperate-tropical comparison, but the evidence is less clear for the subtropical populations without more data on pollination. Future work could investigate how herbivory affects pollination; for example, we have observed *Phytolacca* herbivores consuming flower buds, and fitness impacts of herbivory could manifest through reduced resources available for flowers and fruits.

Although a strength of our study is the analysis of multiple in-depth metrics of plant–insect interactions, a drawback of this design is that it is limited to two species, due to substantial logistical challenges in repeatedly visiting multiple populations across a broad latitudinal range. Because two-species comparisons suffer from a lack of statistical power to form strong conclusions about adaptation (Garland and Adolph 1994), a natural follow up would be to replicate our approach across

several other congeneric species pairs that span tropical and temperate regions to test for generality of the results. Potential New World herbaceous genera for such a study include *Asclepias*, *Cuphea*, *Desmodium*, *Ipomoea*, *Mirabilis*, *Nicotiana*, *Oxalis*, *Passiflora*, *Physalis*, *Ruellia*, *Sida*, and *Solanum*. Despite this limitation, we provide the most comprehensive study to date, addressing several weaknesses of commonly used approaches and recommendations for best practices identified by reviews in the last decade (Schemske et al. 2009, Moles et al. 2011*a*, Anstett et al. 2016), and our results encourage the continued testing of the biotic interactions hypothesis, particularly at large spatial scales.

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SUPPORTING INFORMATION

DATA AVAILABILITY