

Latitudinal variation in herbivory: influences of climatic drivers, herbivore identity and natural enemies

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Although a number of investigations have concluded that lower latitudes are associated with increases in herbivore abundance and plant damage, the generality of this pattern is still under debate. Multiple factors may explain the lack of consistency in latitude–herbivory relationships. For instance, latitudinal variation in herbivore pressure may be shaped entirely or not by climatic variables, or vary among herbivore guilds with differing life-history traits. Additionally, the strength of top–down effects from natural enemies on herbivores might also vary geographically and influence latitude–herbivory patterns. We carried out a field study where we investigated the effects of latitude and climate on herbivory by a seed-eating caterpillar and leaf chewers, as well as parasitism associated to the former across 30 populations of the perennial herb *Ruellia nudiflora* (Acanthaceae). These populations were distributed along a 5° latitudinal gradient from northern Yucatan (Mexico) to southern Belize, representing one-third of the species' latitudinal distribution and the entirety and one-third of the precipitation and temperature gradient of this species' distribution (respectively). We found opposing latitudinal gradients of seed herbivory and leaf herbivory, and this difference appeared to be mediated by contrasting effects of climate on each guild. Specifically, univariate regressions showed that seed herbivory increased at higher latitudes and with colder temperatures, while leaf herbivory increased toward the equator and with wetter conditions. Multiple regressions including temperature, precipitation and latitude only found significant effects of temperature for seed herbivory and latitude for leaf herbivory. Accordingly, that latitudinal variation in seed herbivory appears to be driven predominantly by variation in temperature whereas latitudinal variation in leaf herbivory was apparently driven by other unexplored correlates of latitude. Parasitism did not exhibit variation with latitude or climatic factors. Overall, these findings underscore that the factors driving latitudinal clines in herbivory might vary even among herbivore species coexisting on the same host plant.

A long-standing paradigm in ecology holds that species interactions become increasingly strong towards the tropics, and that this has led to increased diversification rates and thus higher species richness in the tropics (Dobzhansky 1950, Janzen 1970, Schemske et al. 2009). In the case of plant–herbivore interactions, a number of studies have concluded that lower latitudes are associated with increases in herbivore damage (Coley and Aide 1991, Coley and Barone 1996, Hillbrand 2004, Pennings et al. 2009, Schemske et al. 2009, Salazar and Marquis 2012) and plants should have thus evolved higher defences (Rasmann and Agrawal 2011, Pearse and Hipp 2012, Moreira et al. 2014, Pratt et al. 2014). Accordingly, Pearse and Hipp (2012) found that leaf defences in 56 oak *Quercus* species are higher for species growing at lower latitudes, while Rasmann and Agrawal (2011) similarly observed that milkweed *Asclepias* species from lower latitudes are better defended. Nonetheless, there are also several studies that have found no relationship between herbivore damage (or anti-herbivore defences) and latitude (García et al. 2000, Pennings et al. 2001, Moles

and Westoby 2003), a positive relationship (Salgado and Pennings 2005, Garibaldi et al. 2011), or variable relationships depending on the herbivore group considered (Pennings et al. 2009). Accordingly, a recent meta-analysis suggests that negative latitude–herbivory relationships are not as common as previously thought; a meta-analysis by Moles et al. (2011) found that only 37% of the published studies showed higher herbivory at lower latitudes, and the average effect size was not significantly different from zero. As a result, the paradigm of increased herbivory at lower latitudes is under debate.

Multiple factors, many of which have not been usually accounted for, may explain the lack of consistency in latitude–herbivory relationships. First, the strength of herbivore pressure or anti-herbivore defences may depend entirely or not upon abiotic drivers (e.g. temperature, precipitation, seasonality). For example, recent work has shown that large-scale latitudinal variation in herbivore pressure is predominantly explained by climatic variables (Adams and Zhang 2009, Pearse and Hipp 2012, Moreira et al. 2014). Second,

most studies have measured community-level patterns of herbivory by pooling data across groups of herbivores, and the few studies that have looked at damage by individual species or groups have focused on herbivores within the same feeding guild or with a similar diet breadth (but see Andrew and Hughes 2005, Pennings et al. 2009, Anstett et al. 2014). One exception is a study by Pennings et al. (2009) that reported damage separately from chewing, sap-feeding, and gall-making insect herbivores in Atlantic coast salt marshes. This study showed contrasting latitudinal patterns among herbivore guilds, possibly due to different responses to growing season duration and winter conditions in relation to the number of generations per year exhibited by each herbivore group. Variation in latitudinal responses among herbivore guilds may be thus driven by not only to differences in feeding mode or tissue specialization, but also by herbivore life-history traits.

A third factor which may influence latitudinal gradients in herbivore pressure is geographic variation in the strength of top-down control by natural enemies (Dyer 2007, Gripenberg and Roslin 2007, Cornelissen and Stiling 2009, Abdala-Roberts et al. 2010, Mooney et al. 2010). Predator and parasitoid abundance and diversity are thought to be greater at lower latitudes and in warmer climates and thus lead to stronger suppression of herbivores (Dobzhansky 1950, Pennings and Silliman 2005, Van Bael et al. 2008, Schemske et al. 2009, Björkman et al. 2011), but few studies have rigorously addressed the potential effects of climate and latitude on the third trophic level (Björkman et al. 2011). One exception is a study by Stireman et al. (2005) which found that greater climatic variability in temperate relative to tropical regions is associated with decreases in parasitism across numerous caterpillar taxa (Marczak et al. 2011). In contrast, a recent meta-analysis by Mooney et al. (2010) did not find latitudinal variation in bird predation on herbivores or effects of birds on plant damage/growth. Direct effects of variation in climate on plants may also feedback to influence top-down control of herbivores through differences in herbivore diet breadth (Bernays and Graham 1988, Mooney et al. 2012), herbivore susceptibility to predators (Mooney et al. 2012), or predator abundance (Oksanen et al. 1981). Unfortunately, our understanding of the effects of broad-scale climatic variation on predators and parasitoids remains limited, and we largely ignore if the third trophic level modulates latitudinal patterns in herbivory.

The aim of this paper was to examine the effects of latitude and climate on two insect herbivore guilds and natural enemies of one of such guilds. We carried out a field study in which we measured herbivory by a specialist seed-eating caterpillar and a guild of generalist leaf-chewing herbivores, as well as parasitism rates associated to the former across 30 populations of the perennial herb *Ruellia nudiflora* (Acanthaceae). These populations were distributed along a 5° latitudinal gradient (from 16°N to 21°N) extending from northern Yucatan (Mexico) to southern Belize (mean annual precipitation from 700 to 2900 mm, mean temperature from 26 to 24°C, and two-fold seasonality in precipitation), representing one-third of the species' latitudinal distribution (14°N to 29°N) and the entirety and one-third of the precipitation and temperature gradient of this species' distribution (respectively) (Hijmans et al. 2005). Historically,

latitudinal variation in herbivory or defensive traits has been documented across species and comparing temperate versus tropical regions (Coley and Barone 1996, Schemske et al. 2009, Rasmann and Agrawal 2011). However, recent work has demonstrated that smaller-scale latitudinal gradients in climatic variables have shaped intra-specific clines in plant traits (e.g. life history traits and defenses, Woods et al. 2012, Pratt and Mooney 2013), contributing to our understanding of how clinal variation in biotic and abiotic drivers shapes microevolutionary patterns in plants. In the present study, we addressed the following questions: 1) is latitude correlated with the intensity of seed herbivory, leaf herbivory, and parasitism associated to *R. nudiflora*, and do these latitudinal relationships vary in strength and/or direction? 2) which climatic factors explain these latitudinal patterns and do these vary among herbivore guilds or trophic levels? And 3) do latitudinal or climatic clines in parasitism contribute to explain latitudinal variation in seed herbivory? By addressing these questions, our work builds towards an understanding of the joint influences of climatic forcing, herbivore identity, and natural enemies on latitudinal variation in plant-herbivore interactions.

Material and methods

Study system

Ruellia nudiflora is a self-compatible perennial herb distributed from southern United States (Texas) to Honduras (Ortegón-Campos et al. 2012). It grows in disturbed open or partially shaded areas, and under a wide range of climatic and soil conditions (Ortegón-Campos et al. 2012). This species has a mixed reproductive system, producing chasmogamous (CH) flowers that have an open corolla and are visited by pollinators, and cleistogamous (CL) flowers that do not open, have reduced corollas, and self-pollinate obligatorily. Fruits are dry and dehiscent, each one normally bearing seven to 12 seeds that typically fall within a meter of the parent plant after the fruit opens. The peak of CH flower production is during July and August, and CL flower production usually spans over a longer period of time (from May to September, Munguía-Rosas et al. 2012).

Both CH and CL fruits are attacked by larvae of a specialist noctuid moth *Tripudia* sp. (Lepidoptera: Noctuidae) that feeds on seeds prior to fruit dehiscence (Abdala-Roberts and Mooney 2013, 2014). Because the species of *Tripudia* moth has not yet been identified, it is possible that more than one species is feeding on *R. nudiflora*. If so, however, these species are equivalents ecologically because they use the same resource. Adult female moths oviposit on recently pollinated flowers and, unless parasitized, a single larva grows inside a developing fruit, and usually consumes all the seeds (ca 95% of seeds consumed per fruit on average, Abdala-Roberts and Mooney 2013). Previous work has shown that increasing fruit output at the plant level reduces the proportion of fruits attacked per plant (i.e. negative density-dependent attack, Abdala-Roberts and Mooney 2013, 2014). Seed-eating caterpillars are in turn attacked by parasitic wasps belonging to Braconidae (four species), Ichneumonidae (one species), and

Pteromalidae (two species), as well as one fly species belonging to Tachinidae (Abdala-Roberts et al. 2010). Parasitoids stop or reduce seed predator consumption, thus having an indirect positive effect on plant fitness through this so called “seed rescue effect” (Abdala-Roberts et al. 2010). Previous work has shown that parasitoid attack ranges from density-independent (i.e. the proportion of parasitized caterpillars is unrelated to the number of fruits attacked by the herbivore, Abdala-Roberts and Mooney 2013), to weakly positive density-dependent (Abdala-Roberts and Mooney 2014).

Results from a previous study that sampled 21 *R. nudiflora* populations throughout the state of Yucatan (Mexico) showed that there was strong population-level variation in the proportion of *R. nudiflora* fruits attacked by the seed herbivore (up to 8.5-fold, Abdala-Roberts et al. 2010). Likewise, there was even greater population variation for the proportion of seed herbivore larvae parasitized (12-fold, Abdala-Roberts et al. 2010), and as a result of variation in parasitoid attack, there was parallel spatial variation for the indirect effects of parasitoids on plant fitness (13-fold, measured as the proportion of rescued seeds, Abdala-Roberts et al. 2010). Moreover, these large-scale geographical patterns of seed herbivore and parasitoid attack appeared to remain relatively constant across years (Supplementary material Appendix 1 Fig. A1), suggesting that there may be consistent population variation in the structure of interactions throughout the plant’s distribution range in Yucatan. Such a scenario sets the stage for spatially divergent evolutionary outcomes from these tri-trophic interactions (i.e. selection mosaics, Rudgers and Strauss 2004).

Ruellia nudiflora leaves are oval-shaped, arranged in pairs, and are usually found at the base of the plant. Most leaf damage is caused by caterpillars of several species of Lepidoptera. Surveys of leaf consumption conducted on a monthly basis during 2006 and 2007 for *R. nudiflora* populations located in the north, center, and south of Yucatan showed that the most important leaf chewers (in terms of abundance and leaf area consumed) are *Anartia jatrophae* and *Siproeta stelenes* (Lepidoptera: Nymphalidae), which are considered generalist species that feed on several species of Acanthaceae, Scrophulariaceae and Verbenaceae (DeVries 1987, Lederhouse et al. 1992). These two species of caterpillars are responsible for 10–20% of leaf area consumed of *R. nudiflora* and have been recorded in most *R. nudiflora* populations sampled in the Yucatan Peninsula (Ortegón-Campos et al. 2009). Total leaf area lost to insect herbivores in *R. nudiflora* usually ranges from 15–20% (Ortegón-Campos et al. 2009); therefore these two caterpillar species account for most of the leaf herbivory experienced by this plant.

Plant sampling scheme and measurements

We sampled a total of 30 *R. nudiflora* populations distributed along a north-to-south gradient from northern Yucatan (Mexico) to southern Belize spanning ca 900 km and five degrees in latitude (16°N to 21°N, Fig. 1). From north to south along this gradient, there is a four-fold increase in precipitation (700 to 2900 mm per year), a 20% decrease in coefficient of variation in precipitation (among months), and a decrease of 2°C in mean annual temperature (from 26 to 24°C). Importantly, the sampled transect spanned one-third

of the latitudinal distribution range of *R. nudiflora* (14°N to 29°N), and covered the entire precipitation gradient and one-third of the temperature gradient found throughout this species’ distribution. Therefore, our findings are of broad importance for understanding the effects of climatic drivers on latitudinal clines in herbivory and parasitism associated to this plant (Hijmans et al. 2005). Accompanying the sampled climatic gradient are southward increases in primary productivity and plant diversity, and a shift in vegetation type from deciduous spiny tropical forests in the north coast of Yucatan to tropical wet perennial forests in southern Belize (Flores and Espejel 1994). Such clinally-varying biotic and abiotic factors are in turn predicted to correlate with a southward increase in both herbivory and parasitism (Stireman et al. 2005, Rodríguez-Castañeda 2013).

We sampled all 30 *R. nudiflora* populations throughout a two-week period starting in early July 2013. To avoid biases in seed herbivore and parasitoid attack due to population differences in reproductive phenology, we exclusively sampled populations that were at the peak of fruit production (Abdala-Roberts et al. 2010). A total of 10 plants were haphazardly sampled within each population, and for each individual we visually estimated whole-plant leaf damage by insect leaf chewers using the following scale: 0 = undamaged, 1 = 0–25% of leaf area consumed, 2 = 25–50% of leaf area consumed, 3 = 50–75% of leaf area consumed, 4 = more than 75% of leaf area consumed. In addition, for 28 out of the 30 populations sampled for leaf damage, we estimated seed herbivory and parasitism by using 10 different plants to those surveyed for leaf herbivory and collecting 10 mature CH fruits and all mature CL fruits available (usually 3–4 per plant during the sampling period) per plant. Fruits were placed in paper bags and transported to the laboratory where they were dissected to record seed herbivore presence (direct observation of larva or indirect evidence from frass) and parasitoid presence (direct observation of adult or larva or indirect evidence from empty pupal casing) (Abdala-Roberts et al. 2010). Based upon these observations, we calculated the proportion of fruits attacked by the seed herbivore (attacked fruits/total number of fruits collected per plant) and the proportion of seed herbivore-attacked fruits with parasitoid (number of attacked fruits with parasitoid/number of fruits with seed herbivore per plant) (Abdala-Roberts et al. 2010). We calculated these proportions by pooling data across fruit types as previous work has shown that the proportion of attacked fruits per plant does not differ between CH and CL fruits (Munguía-Rosas et al. 2013).

Geographic and climatic variables

The latitude and longitude of each *R. nudiflora* population were acquired in situ using a global positioning system navigation device. To characterize the mean climate of each *R. nudiflora* population site, we used a subset of the BioClim climate variables (available at: <www.worldclim.org/>), namely: BIO1 (annual mean temperature, °C), BIO4 (temperature seasonality, expressed as the standard deviation of temperature among months × 100), BIO5 (maximum temperature of the warmest month, °C), BIO6 (minimum temperature of the coldest month, °C), BIO12 (annual precipitation, mm), BIO13 (precipitation of the wettest month,

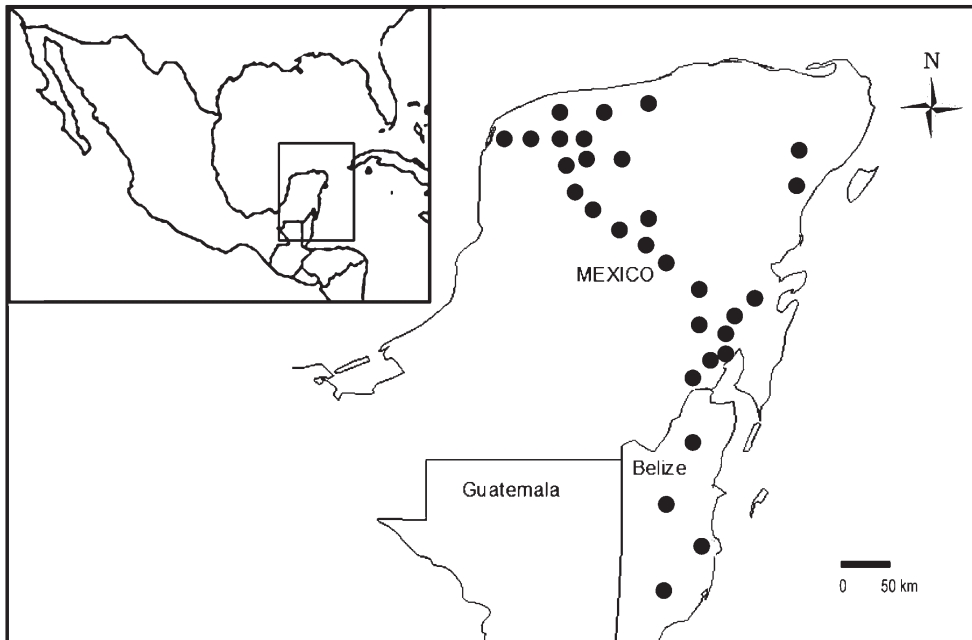


Figure 1. Spatial layout of *Ruellia nudiflora* populations sampled throughout the Yucatan Peninsula (Yucatan and Quintana Roo, Mexico) and Belize. Circles represent the location of each population ($n = 30$).

mm), BIO14 (precipitation of the driest month, mm), BIO15 (precipitation seasonality, expressed as standard deviation of precipitation across months) (Moreira et al. 2014). The procedures used to calculate these variables are fully described in Hijmans et al. (2005).

Statistical analyses

To investigate whether there was population variation in the levels of seed herbivory, leaf herbivory, and parasitism associated with the seed herbivore, we performed general linear models (PROC GLM in SAS 9.2) for each response variable testing for an effect of population which was treated as a fixed factor (*R. nudiflora* populations were intentionally selected in a latitudinal gradient).

To investigate whether latitude and climate were related to seed herbivory (proportion of attacked fruits averaged across plants per population), leaf herbivory (leaf damage score averaged across plants per population), and parasitism intensity associated with the seed herbivore (proportion of attacked fruits with parasitoid averaged across plants per population), we performed simple linear regressions using population means for each variable where latitude or climate were predictors of each type of herbivory or parasitism using PROC REG in SAS 9.2. To make use of the information from all climatic variables without inflating type I error due to multiple tests, prior to running the regressions we summarized climatic variables by conducting two independent principal component analyses (PCA) using PROC FACTOR (rotation = varimax) in SAS 9.2, one for temperature variables and one for precipitation variables (Moreira et al. 2014). In each case, climatic variables were summarized with the first principal component (Moreira et al. 2014). The first principal component explained 79% of the vari-

ance in the four temperature variables across populations ('PC temperature' hereafter), and was positively related to annual mean temperature, temperature seasonality, and maximum temperature of the warmest month, and negatively related to minimum temperature of the coldest month. Similarly, the first principal component explained 77% of the variance in the four precipitation variables across populations ('PC precipitation' hereafter), and was positively related to annual precipitation, precipitation of the wettest month, and precipitation of the driest month. The standardized z -scores of each PC were then used in the regression analyses to examine the relationships between climate and herbivory and parasitism.

Finally, we performed three multiple regressions to simultaneously evaluate the effects of latitude, PC temperature, and PC precipitation on seed herbivory, leaf herbivory, or parasitism. The aim of these regressions was to test whether latitude was a significant predictor of herbivory and parasitism once the effect of climatic factors was accounted for (i.e. if climate explained latitudinal patterns), as well as determine which climatic factor(s) drive(s) variation in herbivory and parasitism once correlations among these factors are accounted for. For all the simple and multiple regressions, we logit-transformed seed herbivory and parasitism data, and log-transformed leaf herbivory data to achieve normality of residuals.

Results

We found population variation in the proportion of attacked fruits by the seed herbivore ($F_{27,286} = 11.74$, $p < 0.001$), in leaf herbivory ($F_{29,220} = 3.01$, $p < 0.001$), and in the proportion of attacked fruits with parasitoids ($F_{27,261} = 4.35$,

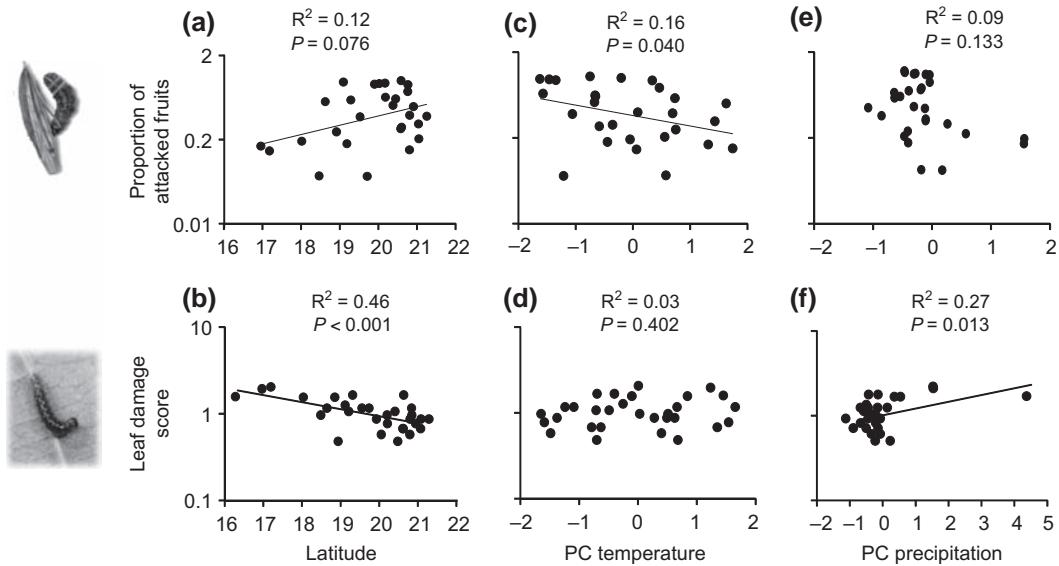


Figure 2. Latitude, temperature, and precipitation as predictors of the proportion of fruits attacked by a Noctuid seed herbivore (a, c, e) and leaf damage by leaf chewers (b, d, f) in *Ruellia nudiflora* populations sampled from northern Yucatan (Mexico) to southern Belize ($n = 28$ populations for seed herbivory and $n = 30$ for leaf herbivory). We plot original values on either a logit (for seed herbivory) or log (for leaf herbivory) scale. R^2 -coefficients and p -values are from simple regressions using transformed data. Circles represent population means ($n = 10$ plants per population).

$p < 0.001$). Latitude was significantly negatively related to PC precipitation ($R^2 = 0.61$, $p < 0.001$) but not to PC temperature ($R^2 = 0.04$, $p = 0.319$), and PC temperature and PC precipitation were unrelated ($R^2 = 0.07$, $p = 0.168$) (Supplementary material Appendix 1 Fig. A2).

The proportion of fruits attacked by the seed herbivore ranged from 0.04 ± 0.02 to 1.00 ± 0.04 (overall mean = 0.43 ± 0.06) among *Ruellia nudiflora* populations. Overall, seed predation was 2.7-fold higher at the northernmost population than at the southernmost population (0.32 ± 0.07 at 21°N versus 0.12 ± 0.04 at 17°N). Simple (univariate) regressions showed that latitude and temperature were associated with seed herbivory across *R. nudiflora* populations marginally significantly (latitude: $R^2 = 0.12$, $p = 0.076$; Fig. 2a) and significantly (PC temperature $R^2 = 0.16$, $p = 0.040$; Fig. 2c), respectively. Specifically, we found that *R. nudiflora* populations from higher latitudes and colder regions (lower mean annual temperature, lower temperature seasonality, and lower maximum temperature of the warmest month based upon the temperature PCA) showed a higher proportion of fruits attacked by the seed herbivore (Fig. 2a, c). Latitude did not significantly predict seed herbivory once climatic factors were accounted for in the multiple regression, and PC temperature was the only significant predictor of seed herbivory in this model (Table 1).

Leaf herbivory scores ranged from 0.5 ± 0.17 to 2.1 ± 0.23 (mean = 1.10 ± 0.08) among *R. nudiflora* populations. Overall, leaf damage was 1.8-fold higher at the southernmost population than at the northernmost population (1.62 ± 0.26 at 21°N versus 0.90 ± 0.18 at 16°N). Simple (univariate) regressions showed that latitude ($R^2 = 0.46$, $p < 0.001$; Fig. 2b) and precipitation ($R^2 = 0.27$, $p = 0.013$; Fig. 2f) significantly predicted leaf herbivory across *R. nudiflora* populations. Contrarily to seed herbivory,

R. nudiflora populations from lower latitudes and wetter regions (higher annual precipitation, higher precipitation of the wettest month, and higher precipitation of the driest month based upon precipitation PCA) showed higher scores of leaf herbivory (Fig. 2b, f), and temperature did not explain population variation in damage by this herbivore guild (Fig. 2d). Multiple regression showed that latitude was the only significant predictor of leaf herbivory and that the climatic correlates of latitude did not explain the latitudinal pattern (Table 1).

The proportion of attacked fruits with parasitoids ranged from 0.07 ± 0.06 to 1.00 ± 0.03 (mean = 0.84 ± 0.04) among *R. nudiflora* populations. Simple regressions showed that neither latitude nor any of the climatic variables were associated with parasitism intensity on the seed herbivore (Fig. 3a–c). These results remained qualitatively unchanged based upon the multiple regression analysis (Table 1).

Table 1. Multiple regression showing the effects of latitude and climate (based upon a principal components analysis summarizing a suite of variables associated to precipitation or temperature) on seed herbivory (proportion of attacked fruits), leaf herbivory (leaf damage score), and parasitism (proportion of attacked fruits with parasitoids) associated with *Ruellia nudiflora*. Student's t -tests (t) and p -values for an analyses based upon logit-transformed (seed herbivory and parasitism) and log-transformed (leaf herbivory) data are shown. Significant ($p < 0.05$) p -values in bold.

Variable	Seed herbivory		Leaf herbivory		Parasitism	
	$t_{1,26}$	p	$t_{1,27}$	p	$t_{1,22}$	p
Latitude	0.37	0.711	-2.14	0.042	0.38	0.706
PC temperature	-2.20	0.038	0.28	0.779	-1.62	0.122
PC precipitation	-0.83	0.416	0.25	0.807	-0.38	0.707

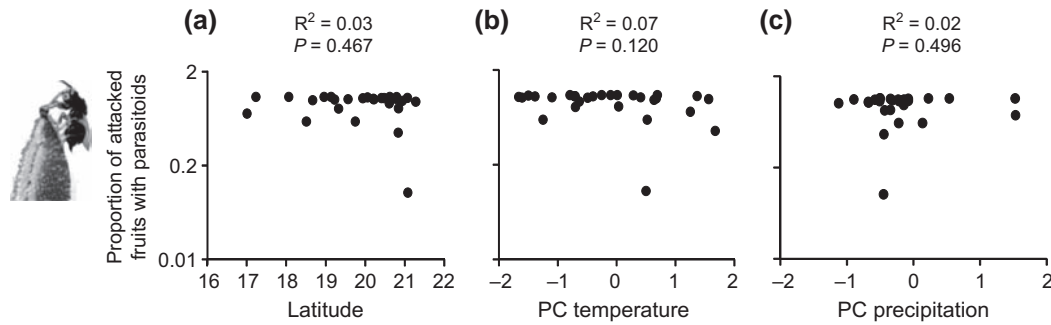


Figure 3. (a) Latitude, (b) temperature and (c) precipitation as predictors of the proportion of attacked fruits with parasitoids across 28 populations of the perennial herb *Ruellia nudiflora* sampled from northern Yucatan (Mexico) to southern Belize. We plot original values on a logit scale on the Y axis. R^2 -coefficients and p-values are from simple regressions using logit-transformed data. Circles represent population means ($n = 10$ plants per population).

Discussion

Latitudinal variation and climatic drivers of seed herbivory and leaf herbivory

Latitude is considered one of the main geographical predictors of species abundance and diversity (Dobzhansky 1950, Janzen 1970). While many studies have supported the hypothesis that herbivory increases with decreasing latitude (Coley and Aide 1991, Coley and Barone 1996, Hillbrand 2004, Pennings et al. 2009, Schemske et al. 2009, Salazar and Marquis 2012), examples of null (García et al. 2000, Pennings et al. 2001, Moles and Westoby 2003) and positive (Salgado and Pennings 2005, Garibaldi et al. 2011) latitude–herbivory relationships are equally and in some cases more common (reviewed by Moles et al. 2011). Accordingly, in this study we found contrasting latitudinal patterns for seed herbivory and leaf herbivory associated with the perennial herb *Ruellia nudiflora*; plant populations from lower latitudes showed higher rates of leaf herbivory but a lower proportion of attacked fruits by the seed herbivore. Because our estimate of leaf herbivory was of low resolution, this finding provides a conservative test for variation among populations and along latitudinal gradients, and it is possible that a more powerful analysis might have also detected effects of precipitation.

One factor possibly underlying the opposing latitudinal gradients in seed and leaf herbivory observed in our study, as well as inconsistent latitudinal patterns from previous studies, is that geographic variation in the strength of herbivore pressure may be shaped entirely or not by climatic variables. Moreover, herbivore species or entire guilds may vary in their responses to different climatic drivers. In our case, multiple regression showed that latitudinal variation in leaf herbivory was not explained by climatic correlates of latitude, i.e. effect of latitude remained significant after accounting for climatic drivers, which themselves did not have an effect (Table 1). This suggests that there were other unaccounted factors that co-vary with latitude and presumably drove the geographical pattern in leaf herbivory. In contrast, and despite sampling a small range in mean annual temperature (2°C , one-third of the temperature gradient of this species' distribution), the only significant predictor of seed herbivory after accounting for all factors in the

multiple regression was temperature. Specifically, we found that *R. nudiflora* populations from colder sites showed a higher proportion of fruits attacked by the seed herbivore. This result is surprising as recent paleobiological studies of the latitudinal diversity gradient have suggested that latitudinal gradients only occur when there is a strong latitudinal climate gradient (reviewed by Mannion et al. 2014). In our case, however, the range in mean annual temperature from the highest to lowest latitudinal point of seed herbivore and *R. nudiflora* distributions is relatively small, spanning only 5°C (from 22°C in southern Texas to 27°C in Honduras). Although theory predicts that both herbivory consumption rates and fitness increase exponentially with increased temperature (O'Connor et al. 2011), insect herbivore performance (e.g. food-to-biomass conversion efficiency) may drastically decline when a species encounters temperatures beyond its thermal optimum (Angilletta 2009, Bauerfeind and Fischer 2013). High temperatures have been also shown to increase the production of plant chemical defences and to reduce nitrogen concentration in plant tissues, diminishing thus plant host quality for herbivores (reviewed by DeLucia et al. 2012). Collectively, our findings suggest that the opposing latitudinal gradients observed for seed herbivory and leaf herbivory in *R. nudiflora* is explained by differences in the identity and relative importance of abiotic (climate) drivers influencing each guild.

Biogeographical and historical factors may also explain the contrasting latitudinal and climatic gradients in seed herbivory and leaf herbivory. Current work suggests that *R. nudiflora* has undergone a recent range expansion from southern Texas into the Gulf of Mexico and southern Mexico (Ortegón-Campos et al. unpubl.), which coincides with increasing human-induced disturbances since the end of the nineteenth century, and start of the twentieth century (Mizrahi et al. 1997). Considering that the *R. nudiflora*-seed herbivore interaction is highly specialized (as observed for other *Tripudia* caterpillars specializing on the genus *Ruellia*, J. Heywood pers. comm., Pogue 2009, as well as based upon previous work in this system, Ortégón-Campos et al. 2009), it is possible that this herbivore has tracked the southward expansion of the distribution range of its host plant. Therefore, the observed increase in seed herbivory with decreasing temperature could reflect a signature of adaptation of this herbivore to its original distribution range at higher

and colder latitudes. In contrast, the most important leaf chewers feeding on *R. nudiflora* are species of Lepidoptera with a wider distribution range and adapted to conditions present in both low and high latitudes (Ortegón-Campos et al. 2012), with warmer temperatures and greater precipitation at lower latitudes likely favouring greater population sizes and increased damage by these herbivores. Additionally, it is also possible that leaf damage patterns might have been driven by unmeasured changes in species richness or composition of generalist leaf chewers, as reported by a recent study (Salazar and Marquis 2012).

Another plausible reason for the contrasting latitudinal patterns of seed and leaf herbivory could be that geographical clines in plant–herbivore interactions vary with herbivore identity depending on the feeding guild, diet breadth, or life-history traits (Salazar and Marquis 2012). Accordingly, Pennings et al. (2009) observed that plant damage by chewing and gall-making herbivores was greater at low-latitude sites than at high-latitude sites in Atlantic Coast salt marshes, whereas damage by sap-feeding herbivores was unrelated to latitude. The authors speculated that univoltine populations of chewing herbivores are less abundant and cause less damage at higher latitudes because they are more heavily impacted by the short growing season and harsh environmental conditions during the winter present in more northern sites (Pennings et al. 2009). In contrast, sap-feeding herbivores such as aphids have multiple generations per year, and their populations can therefore recover more easily from winter losses (Pennings et al. 2009). Additionally, with respect to herbivore diet breadth, it has been predicted that specialist and generalist herbivores are differentially adapted to plant defences (Leimu et al. 2005, Mathur et al. 2011, Ali and Agrawal 2012). While specialist herbivores are better-adapted to qualitative defences such as alkaloids, generalist herbivores are better-adapted to quantitative defences such as phenolic compounds (Leimu et al. 2005, Mathur et al. 2011, Ali and Agrawal 2012). Because quantitative and qualitative defences may shape variation in the strength or direction of herbivory–latitude relationships (Marquis et al. 2012, Pearse and Hipp 2012), and at the same time may exhibit different relationships with latitude, it is thus reasonable to expect that damage by herbivores with contrasting diet breadths might vary in their relationship with latitude. Accordingly, further research is needed to determine whether qualitative (i.e. alkaloids) and quantitative (i.e. phenolics) defences in seeds and leaves of *R. nudiflora* populations vary with the latitude and explain the observed latitudinal patterns for seed herbivory (specialist) and leaf herbivory (generalists).

Geographic variation in parasitoid attack

Parasitism intensity associated with the seed herbivore was extremely high with 84% of the attacked fruits having parasitoids, on average, and 24 out of 28 populations exhibiting greater than 70% parasitism. However, despite observing a large amount variation in the strength of top-down effects by parasitoids, and contrary to our expectations, our results showed that parasitism intensity was unrelated to latitude or climatic conditions. Accordingly, this result suggests that geographic variation in parasitism rates does

not contribute the observed latitudinal and climatic gradients in *R. nudiflora* seed herbivory throughout the sampled latitudinal gradient. These results run counter to previous investigations reporting that top-down effects of the third trophic level on insect herbivores are greater in warmer, less seasonal climates, which are characteristic of lower latitudes (Coley and Aide 1991, Novotny et al. 1999, Heil 2008). The higher temporal stability in climatic conditions at lower latitudes have been proposed to increase predator and parasitoid effects by increasing the predictability of host traits and population dynamics (Stireman et al. 2005). Additionally, tropical plants may be better defended against herbivores than temperate species, reducing herbivore performance (Salazar and Marquis 2012), which in turn leads to slower herbivore growth rates and thus heightened risk of predation or parasitism (slow-growth, high-mortality hypothesis; reviewed by Williams 1999). While these studies provide good evidence for the linkage between climate and predation/parasitism rates as well as latitudinal variation in natural enemy effects, our results highlight the importance of considering system-to-system variation and to account for specific factors which could be driving the observed patterns (Zhang and Adams 2011). For example, dietary herbivore specialists are predicted to be superior to generalists at exploiting their host plants for defence or refuge from enemies, leading to weaker top-down effects of the latter (Bernays and Graham 1988; Singer et al. 2014). Accordingly, further work is needed to determine which herbivore and natural enemy traits underlie geographical variation in tri-trophic interactions as these may cause departures from the expected patterns.

Conclusions

By sampling a substantial portion of the latitudinal gradient and most of the climatic variation of the distribution range of the perennial herb *R. nudiflora*, we show that latitudinal clines in plant–herbivore interactions are contingent upon climatic effects and herbivore identity. These findings call for further work examining latitudinal patterns of herbivory across multiple herbivore species varying in traits such as feeding guild and dietary specialization in order to gain a predictive understanding of how herbivore traits interact with climate in predicting geographical gradients in herbivory. Finally, contrarily to evidence gathered thus far, our work showed that climate and latitude do not have effects on parasitism intensity, in turn suggesting that natural enemies do not have a predominant role in shaping geographical gradients of seed herbivory associated with *R. nudiflora*. Nonetheless, further work examining latitudinal variation in tri-trophic interactions is much needed. Overall, this study lays a foundation towards understanding the simultaneous influence of climatic forcing and natural enemies on geographic variation in plant–herbivore interactions.

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Supplementary material (available online as Appendix oik.02040 at <www.oikosjournal.org/readers/appendix>). Appendix 1.