

# Ecological and evolutionary consequences of plant genotype diversity in a tri-trophic system

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**Abstract.** The mechanisms by which plant diversity influences consumers are largely unexplored. Plant diversity reduces among-plant competition, and as a result, may have cascading bottom-up effects through altered resource quantity and quality. Less understood are parallel effects of resource heterogeneity on consumer foraging behaviors, and the consequences of such effects for trophic interactions and feedbacks on plant performance. Here, we asked whether genotypic diversity in the herb *Ruellia nudiflora* influences seed predator (SP) and parasitoid functional responses, and if such effects in turn influence selection on the plant. We established plots with plants of one or five genetic families (pool = 14) and measured fruit, seed predator, and parasitoid abundance. By eliminating direct plant-plant interactions, this experiment explicitly tested for plant diversity effects occurring through altered consumer behaviors (vs. effects through changes in plant quantity or quality). We compared observed plant fitness (under three trophic levels) to projected fitness in the absence of parasitoids (two trophic levels) and in the absence of seed predation (one trophic level) by computing the number of seeds consumed by the SP and the number of seeds rescued from consumption by parasitoids. We then compared the strength and mode of selection on fruit number between levels of diversity, separately under each trophic scenario. Plant diversity did not influence fruit, seed predator, or parasitoid abundance, but did alter plant-SP interactions. SP recruitment resembled a Type II functional response but saturated weakly in polycultures, with fewer SPs at intermediate fruit abundance and more SPs at high fruit abundance relative to monoculture. Parasitoid recruitment was weakly positively density dependent and unaffected by diversity. Importantly, we found that under the bi-trophic scenario, the effect of diversity on SP recruitment altered the mode of selection on fruit number, from directional selection at low diversity to nonlinear (stabilizing) selection at high diversity. In contrast, diversity did not alter selection under mono- or tri-trophic scenarios. Therefore, diversity effects on SP functional responses fed back to alter selection on fruit number, but parasitoids eliminated this linkage by weakening herbivore selection. Collectively, these findings provide novel evidence for the mechanistic basis of eco-evolutionary feedbacks between plant diversity and consumers.

**Key words:** *feedback; genotype diversity; interaction modification; Ruellia nudiflora; seed predator-parasitoid; tri-trophic interactions.*

## INTRODUCTION

As a result of more than two decades of research, there is now widespread support for the effects of plant diversity on ecosystem processes and community structure at higher trophic levels (Johnson et al. 1996, Cardinale et al. 2004, 2011, Hooper et al. 2005). Studies have found that higher plant species diversity promotes greater primary productivity (Johnson et al. 1996, Tilman et al. 1996), enhances stability in ecosystem processes (Naeem et al. 1994, Cottingham et al. 2001, Tilman et al. 2006), and influences arthropod community structure (Siemann 1998, Haddad et al. 2009,

Scherber et al. 2010). More recently, the role of intraspecific plant diversity, frequently measured as plant genotype richness, has emerged as a parallel and equally important component of plant diversity (Hughes et al. 2008, Bailey et al. 2009, Bolnick et al. 2011), with work showing that this source of plant diversity can have effects of similar magnitude or even greater than species diversity effects (Crawford and Rutgers 2013).

Both plant inter- and intraspecific diversity effects are assumed to operate through some combination of complementarity (i.e., facilitation, niche partitioning [Loreau and Hector 2001, Tilman et al. 2002]) and sampling effects (i.e., chance of including highly performing species or genotypes is greater at high diversity [Houston 1997, Tilman et al. 2002]). Whether through complementarity or sampling effects, the influence of diversity on plant growth (and potentially also quality [McArt and Thaler 2013]) is in turn expected

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to cascade up and influence higher trophic levels. Accordingly, there is widespread evidence for positive effects of plant intra- and interspecific diversity on producer biomass influencing arthropod abundance and community structure (Siemann 1998, Crutsinger et al. 2006, Cook-Patton et al. 2011, McArt et al. 2012).

Despite increasing recognition for the influence of plant diversity on higher trophic levels, very few studies have addressed the mechanisms for such effects (Rafaeli et al. 2002, Moreira et al. 2012, Moreira and Mooney 2013). Studies that have looked at the effects of plant diversity on consumers have usually documented broad changes in community structure (Siemann 1998, Crutsinger et al. 2006, 2008, Johnson et al. 2006, Haddad et al. 2009, Scherber et al. 2010), while comparatively less attention has been given to changes in focal interactions (but see Ninkovic et al. 2011, McArt and Thaler 2013, Moreira and Mooney 2013). As a result, we currently lack a predictive understanding of how plant diversity effects on species interactions scale-up to influence community structure (Johnson 2008), as well as the relative importance of different mechanisms by which plant diversity influences consumers (Mooney and Singer 2012).

One approach for assessing the mechanisms by which plant diversity influences interactions at higher trophic levels is determining if such effects are density- or trait-mediated (Mooney and Singer 2012). In the first case, plant diversity may drive an increase in plant biomass, which in turn causes greater herbivore abundance, and potentially also greater predator abundance (i.e., an “interaction chain” sensu Wootton 1994). Here, the function describing pairwise (e.g., herbivore–carnivore) interactions, which can be linear or nonlinear, remains unchanged (Mooney and Agrawal 2008, Mooney and Singer 2012, Singer et al. 2012). There is good evidence for this mechanistic pathway from studies showing that greater plant biomass at high diversity results in parallel increases in the abundance of herbivorous and predatory arthropods (Crutsinger et al. 2006, Haddad et al. 2009, Cook-Patton et al. 2011, Genung et al. 2011, McArt et al. 2012). Accordingly, density-mediated effects are assumed to be a widespread mechanism by which plant diversity influences associated communities (Cook-Patton et al. 2011).

Alternatively, plant diversity may alter the function describing pairwise species interactions through interaction modifications (sensu Wootton 1994). Here, plant diversity may influence the strength or function of interactions through effects on the traits or behaviors of one or both interacting species via increased resource heterogeneity (e.g., Moreira et al. 2012, Moreira and Mooney 2013). One of the few examples testing for this mechanism is a study by Moreira et al. (2012) showing that recruitment of predatory ants occurred at a greater rate in patches with high plant diversity, resulting in stronger positive indirect effects of ants on plant growth (i.e., a feedback between bottom-up effects of producer

diversity and top-down effects of carnivores). Unfortunately, plant diversity research has lacked an explicit consideration of interaction modifications or separated them from density-mediated effects (Moreira and Mooney 2013).

Separating density-mediated effects from interaction modification effects is important because it enables one to assess the ecological and evolutionary consequences of plant bottom-up effects on species interactions (Abrams 1995, Strauss et al. 2005, Mooney and Singer 2012). In particular, changes in the strength of species effects through interaction modifications are recognized as a primary source of evolutionary change due to alteration of selection on species traits (Miller and Travis 1996, Inouye and Stinchcombe 2001, Mooney and Singer 2012). Moreover, the few studies available (e.g., Moreira et al. 2012) suggest that feedbacks between plant diversity and consumer effects may have important, but currently unexplored, evolutionary consequences. Accordingly, addressing interaction modifications (as opposed to density-mediated effects) will enable us to assess the evolutionary consequences of plant diversity effects via altered trophic interactions as well as eco-evolutionary feedbacks (Agrawal et al. 2006, Parker et al. 2010, Genung et al. 2011, Utsumi et al. 2011).

*Ruellia nudiflora* is a short-lived perennial herb found throughout southern Mexico. Its fruits are attacked by larvae of a seed-eating moth, which in turn is parasitized by several species of wasps that reduce herbivore seed consumption (Abdala-Roberts et al. 2010; see Plate 1). The seed predator forages in a negative density-dependent manner, where the proportion of attacked fruits per plant decreases with increasing fruit output (Abdala-Roberts and Mooney 2013). A previous experiment with this system showed that the seed predator selects for greater fruit number, while parasitoids in turn weaken this selective effect (Abdala-Roberts et al. 2014). Here we report on an experiment designed to test for the bottom-up effects of plant genotype diversity on these trophic interactions, and how such effects in turn alter seed predator and parasitoid top-down selective impacts on the plant. We eliminated plant–plant interactions (above and below ground) in order to specifically address the bottom-up effects of resource diversity arising through altered consumer foraging behaviors (Agrawal et al. 2006), while controlling for the effects of diversity on plant quality or quantity that have been identified in other systems (e.g., Crutsinger et al. 2006, Haddad et al. 2009, Cook-Patton et al. 2011, McArt and Thaler 2013). In addition, plant genotypes used in this study originated from the same source population, allowing for a realistic assessment of the selective impacts of altered species interactions based upon natural levels of intra-population genetic variation exhibited by *R. nudiflora* (Tack et al. 2012).

With this system we first asked: (1) whether plant genotype diversity (hereafter plant “diversity”) influ-

enced consumer abundance and modified the function of plant–seed predator and seed predator–parasitoid interactions, while controlling for density-mediated effects from increased plant biomass by preventing interactions among neighboring plants. And (2), whether any such alteration of consumer functional responses fed back to influence selection on plant traits. We hypothesized that plant diversity effects on plant–seed predator interactions would in turn alter selection imposed by seed predators on fruit number because of changes in the relationship between the proportion of attacked fruits and fruit number. Moreover, we expected that parasitoid top-down effects would weaken the linkage between diversity and herbivore selection because parasitoids reduce seed predator consumption, and in so doing, dampen their selective effects on fruit number. This work is novel in that it addresses the mechanisms by which plant diversity influences resource–consumer interactions, as well as eco-evolutionary feedbacks stemming from such dynamics and influencing plant evolution (Agrawal et al. 2006, Post and Palkovacs 2009, Genung et al. 2011).

## METHODS

### *Natural history*

*Ruellia nudiflora* Engelm. and Gray Urb. (Acanthaceae) is distributed from Texas (USA) to Honduras (Long 1977), and in Yucatán (southeast México) it grows across a wide range of abiotic conditions (Ortegón-Campos et al. 2012). This species is self-compatible and produces chasmogamous (CH) flowers that have an open corolla ( $2.33 \pm 0.04$  cm in diameter [mean  $\pm$  SE; data from the present study] and are visited by insect pollinators, and cleistogamous (CL) flowers that do not open, have reduced corollas ( $0.25 \pm 0.01$  cm in diameter; data from the present study), and self-pollinate obligately. Chasmogamous flowers may also self-pollinate autonomously upon corolla dehiscence when anthers come into contact with the stigma (Abdala-Roberts et al. 2014). Chasmogamous flowers are found on vertical inflorescence stalks that are 20–30 cm in height, while CL flowers are usually produced on lateral inflorescences at the base of the plant. In addition, CH flower production occurs during July and August and may overlap to some extent with CL flowering, although the latter spans a longer period of time (Munguía-Rosas et al. 2012). Fruits are dry and dehiscent and seeds disperse ballistically; CH fruits are larger and produce more seeds (CH fruits,  $13.27 \pm 0.16$  seeds [mean  $\pm$  SE]; CL fruits,  $9.44 \pm 0.02$  seeds) (Abdala-Roberts and Mooney 2013). On average, chasmogamous fruits weigh  $28.74 \pm 0.45$  mg, and CL fruits weigh  $16.61 \pm 0.22$  mg (Munguía-Rosas et al. 2013). Reproduction of young (i.e., first-year) plants is skewed towards CL reproduction (75–85% of fruits produced by first-year plants are CL [Abdala-Roberts and Mooney 2013; data from this study]), whereas older plants invest proportionally the same in each reproduc-

tive function (Munguía-Rosas et al. 2012). Genetic variation for CL fruit number is large (>3-fold [Abdala-Roberts and Mooney 2013]), suggesting that trade-offs (e.g., current vs. future reproduction) maintain variation in reproductive output in this species.

Both CH and CL fruits are attacked by larvae of a single, as yet unidentified species of noctuid moth (Lepidoptera: Noctuidae). Although evidence thus far suggests that only one seed predator species is present in this system (based on sampling from more than 20 populations [Abdala-Roberts et al. 2010]), the possibility of having cryptic species cannot be discarded. Nonetheless, if cryptic species do occur, they are likely equivalents ecologically because they cannot be distinguished morphologically and use the same resource. Female moths oviposit on recently pollinated flowers, and unless parasitized, a single larva grows inside a developing fruit and usually consumes all the seeds prior to fruit dehiscence (95%  $\pm$  1.0% of seeds consumed per fruit on average [Abdala-Roberts and Mooney 2013; data from this study]). Larval development takes place within a single fruit (one larva per fruit), and larvae usually do not move among fruits (L. Abdala-Roberts, *unpublished data*). Previous work has shown that fruiting synchrony at the patch level (V. Parra-Tabla, *unpublished data*) and fruit output at the plant level (Abdala-Roberts and Mooney 2013, Abdala-Roberts et al. 2014) reduce the proportion of fruits attacked per plant (i.e., negative density-dependent attack). Accordingly, these studies have demonstrated that recruitment of seed predators to fruits is a nonlinear relationship estimated by a quadratic regression model where both fruit number and (fruit number)<sup>2</sup> are significant predictors of seed predator abundance (Abdala-Roberts and Mooney 2013, Abdala-Roberts et al. 2014), and where a quadratic model explains more of the variation in seed predator abundance and provides a better fit (based upon  $R^2$  and AIC, respectively) than a linear model (data from this study). Seed predator larvae are in turn attacked by seven species of parasitic wasps belonging to Braconidae (four species), Ichneumonidae (one), and Pteromalidae (two), as well as one fly species belonging to Tachinidae. 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). Both seed predation and parasitism intensity (and thus the top-down effect of parasitoids on plant fitness) vary across plant populations (Abdala-Roberts et al. 2010).

### *Experimental design and sampling*

The first week of July 2012, we collected 10–15 CH fruits from each of 14 plants of a source population located 12 km south of Mérida, Yucatán (México). All seeds from a given plant were considered a maternal family composed of a mixture of full- and half-sibs, and were germinated immediately after collection. Mother plants were sampled from the same population in order

to assess patterns of selection on plant traits based upon realistic (natural) levels of genetic variation for this species (Tack et al. 2012). One month after germination (mid-August 2012), seedlings were transplanted to plastic 1-L growing bags filled with a 1:1 mix of native soil and peat moss, and remained in a nursery for a two-month period under homogeneous environmental conditions to reduce maternal effects. During this period, each plant was fertilized once with 40 mL of a solution (Ferticoral, Impulsora Agroquímica del Sureste, Mérida, Mexico) containing N (20%), P (30%), and K (10%), at a concentration of 2 g/L.

In late September 2012, plants were transported to a site located 4.6 km east of the town of Molas, Yucatán (México) ( $20^{\circ}29'10''$  N,  $89^{\circ}59'75''$  W), and 7 km southwest of the population source site. A total of 63,  $1 \times 1$  m plots were established throughout a 600-m<sup>2</sup> area where the vegetation was composed of small shrubs, herbs, and grasses, and surrounded by a matrix of secondary tropical forest. Each plot contained five plants arranged in an 80 cm diameter circle, with 20–25 cm spacing between plants ( $n = 315$ ). This spacing resulted in a density of plants similar to that found within *R. nudiflora* populations, and is thus expected to have realistic effects on consumers. In addition, plot size was chosen based upon previous work assessing variation in seed predator attack rates at the patch level and showing that this spatial scale is relevant to seed predator foraging patterns and recruitment to fruits (Cuautle and Parra-Tabla 2014; see Bommarco and Banks 2003, Hämbäck et al. 2009). Of the total number of plots established, 28 represented monocultures where all plants belonged to the same family (two replicate plots per family), while 35 plots represented polycultures where each plant belonged to a different family. Although the genotype composition of polycultures was determined by assigning random combinations of five plant families (out of 14 possible) to each plot, all genotypes were represented approximately equally in polyculture plots (range 11–13 occurrences per genotype). Monoculture and polyculture plots were interspersed throughout the study site and arranged in blocks of four plots each, and in all but three blocks the number of plots of each level of diversity was the same (i.e., two plots of each level within each block). The block effect was included to account for environmental heterogeneity previously shown to influence species interactions associated with *R. nudiflora* (e.g., light availability [Munguía-Rosas et al. 2013]). Mean distance among plots within each block was 2 m, while mean distance between adjacent blocks was 4 m.

When planted in the ground, plants were left in the plastic bags they were grown in to prevent underground plant–plant interactions. At the time of planting, we cut the bag on one side to prevent root binding and oriented the bag such that the slit was facing outward, thus impeding root contact with adjacent plants. At the conclusion of the experiment, we excavated 20% of the

plants (three randomly chosen plants from 20 plots) and confirmed that in all cases roots between adjacent plants were not in contact. The amount of spacing among plants also prevented aboveground interactions due to canopy contact or shading. Overall, this approach was aimed at controlling for above- and underground interactions among neighboring plants influencing biomass production (and potentially also plant quality to herbivores [McArt and Thaler 2013]), while testing exclusively for interaction modifications due to resource or habitat heterogeneity (e.g., plant apparency, chemical or physical interference [Andow 1991, Plath et al. 2012, Castagneyrol et al. 2013]).

Twice a week, from early October 2012 to late December 2012, we collected all mature fruits produced per plant (CH and CL) and dissected them under a microscope to record seed predator and parasitoid attack, as well as seed number. For each plant, we summed the total number of sampled fruits (i.e., estimate of total fruit production), fruits attacked by the seed predator, and attacked fruits with a parasitoid (i.e., number of parasitized herbivores), in all cases separately for each fruit type. Subsequently, these values were summed for each plot to estimate plot-level CL and CH fruit number, seed predator number, and parasitoid number. Calculations were based upon fruit and insect abundance data pooled across all sampling periods, and in the case of parasitoid abundance, we also pooled specimens across species.

#### *Mechanisms of diversity effects on consumer abundance and interactions*

First, we tested for an overall effect of diversity (fixed effect, two levels) on fruit, seed predator, and parasitoid number at the plot level using general linear models (hereafter “initial” models). Each model also included the effect of fruit type (fixed effect, two levels), block (random effect), and plot (random effect, nested within block); fruit type was included to account for fruit dimorphism in this species, but was not used to test any underlying hypothesis, while plot was included to account for nonindependence of the two fruit samples (one per fruit type) drawn from each plot. In this analysis, any diversity effects on fruit output would be exclusively due to sampling effects (i.e., occurrence of plant families with greater reproductive output is more likely at high diversity [Houston 1997, Tilman et al. 1997]), as complementarity effects via plant–plant interactions (i.e., niche partitioning [Loreau and Hector 2001, Tilman et al. 2002]) were prevented by the experimental procedure which restricted below- and aboveground interactions among plants. Accordingly, to test for sampling effects we used standard methods (Loreau and Hector 2001) and found that such effects on fruit, seed predator, and parasitoid abundance were not statistically different from zero (see *Results*). Therefore, a positive effect of diversity on insect abundance would not be due to increased fruit number

from either complementarity or sampling effects (see *Results*), but rather due to altered species interactions (e.g., differences in rates of seed predator or parasitoid recruitment).

To assess if plant diversity mediated plant–seed predator and seed predator–parasitoid interactions, we departed from the initial insect abundance models and constructed (hereafter “mechanistic”) models with additional terms to describe consumer functional responses, and the dependency of such responses on diversity. For the seed predator model, we included fruit number and (fruit number)<sup>2</sup> to test for both linear and nonlinear responses (respectively) to resource abundance, as well as the fruit number × diversity and (fruit number)<sup>2</sup> × diversity interactions. Both of these interaction terms tested for a change in the function of plant–seed predator interactions between levels of diversity after accounting for fruit number (i.e., an interaction modification). Including (fruit number)<sup>2</sup> tests whether the relationship between fruit number and seed predator number is nonlinear (i.e., curved), having accounted for the linear relationship based upon the linear term for fruit number. Likewise, the parasitoid model was parallel to that for seed predators, except that instead of fruit number it included both the linear and quadratic terms for seed predator number, which is the resource to which these consumers respond to, as well as the seed predator number × diversity and (seed predator number)<sup>2</sup> × diversity interactions. As just described, including (seed predator number)<sup>2</sup> tests whether the relationship between parasitoid number and seed predator number is nonlinear (i.e., curved), having accounted for the linear relationship based upon the linear term for seed predator number.

Testing for the significance (or contribution) of the quadratic term (and the interaction including this term) in these mechanistic models could also be done through information criteria-based model selection, comparing models with and without the quadratic term. Comparisons based upon AIC values did not suggest model fits were improved with the inclusion of the quadratic term in the instance where the *P* value for the quadratic term was significant. While there is discrepancy between these two legitimate approaches, here we base our conclusion upon the significance of the *P* values for the effect of the quadratic term and the interaction including this term, a conclusion that is also supported by the fact that two past studies from this system have similarly found nonlinear functional responses (Abdala-Roberts and Mooney 2013, Abdala-Roberts et al. 2014).

Both initial and mechanistic models were performed with PROC MIXED in version 9.2 (SAS 2008), under the assumption of normally distributed residuals, which was met in all cases (based on inspection of residuals and Shapiro-Wilk tests). We did not test for significance of random effects, since these terms did not address any of our predictions and were instead used to account for spatial variation in interactions (block effect) and

nonindependence of data collected from the same plot (plot effect). For both types of models, we present least-square means ± SE as descriptive statistics. In the case of sampling effects of diversity, we present values and confidence intervals.

#### *Evolutionary consequences of plant diversity effects on interactions*

If genotype diversity altered the function of consumer interactions, such effects may in turn have consequences for the evolution of plant traits influencing such interactions (Agrawal et al. 2006). Specifically, we hypothesized that changes in the function of plant–seed predator interactions (i.e., change in the rate of seed predator recruitment to fruits) between monocultures and polycultures would lead to differences in the strength or mode of selection by the seed predator on fruit number. Moreover, because parasitoids have previously been found to dampen selection by the seed predator on fruit number (Abdala-Roberts et al. 2014), we expected that this top-down effect would influence the linkage between diversity and herbivore selection. All selection analyses described in the following sections were performed by pooling CH and CL fruits, as data from this experiment showed that the function of plant–seed predator and seed predator–parasitoid interactions did not vary between fruit types (non-significant fruit number × fruit type [ $F_{1,48} = 1.50, P = 0.22$ ] and seed predator number × fruit type [ $F_{1,50} = 1.00, P = 0.32$ ] terms, from the seed predator and parasitoid abundance models, respectively). Data from this study also showed significant (close to threefold) variation in fruit production (CH and CL fruits pooled) among plant families ( $10.64 \pm 1.85$  fruits to  $29.40 \pm 3.72$  fruits;  $F_{13,301} = 3.07, P = 0.003$ ), suggesting ample genetic variation in this trait for consumers to select upon. Although positive selection on fruit number is expected (i.e., this trait is a component of fitness), and seed number per fruit is relatively conserved and varies little among plant families, the large genetic variation in fruit number suggests the presence of trade-offs between current and future reproduction, which could lead to variation in selection on fecundity in *R. nudiflora* (Abdala-Roberts and Mooney 2013).

*Plant fitness under different trophic scenarios.*—To disentangle the effects of seed predators, parasitoids, and plant diversity on selection on fruit number, we calculated plant fitness under three trophic scenarios following the procedures described in Abdala-Roberts et al. (2014). Experimentally excluding seed predators and parasitoids to test for their individual and combined effects on plant fitness is not feasible in this study system; seed predators cannot be excluded without affecting pollinators, and parasitoids cannot be excluded without affecting seed predators and pollinators. Yet the nature of this system makes it possible to measure the impacts of seed predators and parasitoids without experimental manipulation. First, we estimated poten-

tial seed number in the absence of seed predator effects (hereafter “mono-trophic” scenario) by multiplying the number of fruits produced per plant (i.e., based on the sum of weekly counts) by the corresponding plant family mean for seed number in unattacked fruits (fruit size is correlated with seed number and there is no detectable difference in the size of fruits attacked and not attacked by seed predators [Abdala-Roberts and Mooney 2013]). Second, fitness in the presence of seed predators alone (hereafter “bi-trophic” scenario) was estimated by assuming that all seeds rescued by parasitoids were instead lost to herbivory (potential seed number – seeds consumed assuming no seed rescue). Results did not change qualitatively after accounting for rare cases of remaining seeds in fruits with unparasitized herbivores (5% of all seeds recorded); thus for simplicity we assumed that all seeds were consumed in all fruits attacked by the seed predator with no evidence of parasitism. Third, the observed number of seeds per plant represented fitness in the presence of both seed predators and parasitoids (hereafter “tri-trophic” scenario). In all three cases, estimates of seed number were based upon values specific to each fruit type, but fitness values were subsequently pooled across fruit types for analyses. We then computed fitness means for each family under each trophic scenario separately for high- and low-diversity treatments, and calculated relative fitness by dividing these values by overall mean fitness across families specific to each trophic scenario and level of diversity. Importantly, previous work has shown that seed predator and parasitoid attack vary greatly across *R. nudiflora* populations (5–60% and 0–65%, respectively;  $N = 21$  populations [Abdala-Roberts et al. 2010]). Therefore, these trophic “treatments” represent realistic scenarios of spatial variation in the strength of tri-trophic interactions, including populations where both seed predation and parasitism are low (mono-trophic scenario), others where seed predation is high but parasitism is low (bi-trophic scenario), as well as cases where both seed predation and parasitism are high (tri-trophic scenario [Abdala-Roberts et al. 2010, Cuautle and Parra-Tabla 2014]). We used plant family means in all selection analyses because genotypic selection analysis avoids environmentally induced correlations between measured and unmeasured traits that can influence estimates of plant fitness in phenotypic selection analyses (Rausher 1992, Stinchcombe et al. 2002).

Our assumption in these fitness estimates is that there is no resource reallocation from damaged fruits. If this is not the case, then seed predator impacts on plant fitness may be overestimated if plants reduce resource investment in damaged fruits, with this in turn increasing investment in undamaged fruits or subsequent fruit production. There are two possible mechanisms for this. First, maturation of damaged fruits might be halted via selective fruit abortion (e.g., Östergård et al. 2007); and second, damaged fruits might have fewer or smaller

seeds (though previous work in other systems has shown seed predator or frugivore bias towards larger, more seeded fruits; e.g., Herrera 2000). We offer three lines of evidence that suggest that resource reallocation effects were weak or absent in this system. First, while *R. nudiflora* has been shown to abort fruits under conditions of low light availability (Abdala-Roberts et al. 2014), plots were placed at full sunlight and we did not find evidence of fruit abortion throughout the duration of the experiment. We assessed this by marking all CH flowers produced by all plants throughout the first month of the experiment. Because a proportion of these fruits were attacked, this suggests that seed predator damage (or other factors) did not induce selective fruit abortion. Second, fruit size of damaged and undamaged fruits was statistically indistinguishable ( $F_{1,202} = 2.98$ ,  $P = 0.10$  [Abdala-Roberts and Mooney 2013]), and this trait is correlated with seed number (Pearson’s  $r = 0.45$ ,  $P < 0.001$ ). Although it is possible that fruit elongation was not affected by seed predator damage, but that seed production and maturation were reduced, this suggests that resources were not shunted from attacked fruits, as this could have caused a reduction in fruit size. Third, for each family we related the mean number of seeds per undamaged fruit per plant to the proportion of attacked fruits per plant. If resources were diverted from damaged to undamaged fruits, then plants with a greater proportion of attacked fruits should have undamaged fruits with more seeds. However, for all families we found either no relationship ( $R^2$  values ranging from 0.0006 to 0.15) or a negative relationship (four families,  $R^2$  values ranging from 0.26 to 0.66). Moreover, the cases of negative relationships instead suggest that negative effects of seed predators on plant fitness may extend beyond seed consumption if plants sustaining higher herbivore loads undercompensate in terms of seed production in undamaged fruits or subsequent fruit production (i.e., reduced tolerance). Although these findings collectively suggest that resources were not re-allocated away from damaged fruits, thus supporting our estimates of seed predator (and parasitoid) impacts on plant fitness, we cannot rule out that seed predator attack causes other (unmeasured) shifts in resource allocation patterns (e.g., between growth and defense).

**Selection analyses.**—To quantify patterns of selection separately for each level of diversity and trophic scenario, we estimated directional selection on fruit number using simple linear regressions where relative fitness was predicted by fruit number (Lande and Arnold 1983), separately for each trophic scenario within each level of diversity (i.e., six estimates). We then repeated the same set of regression models, including both the linear (fruit number) and quadratic ( $[fruit\ number]^2$ ) terms, the latter of which tests for nonlinear (i.e., stabilizing or disruptive) selection. Following the convention for regression-based selection analyses, directional selection was first estimated using

the simple linear model, and nonlinear selection was subsequently estimated based upon a model with both the linear and quadratic terms (Fairbairn and Preziosi 1994; for examples see Juenger and Bergelson 1998, Pilson 2000). Although the linear term was included in both the linear and quadratic models, directional selection was estimated using only the linear model, because this is considered to provide a better estimate of directional selection than the linear term from a quadratic model (Scheiner et al. 2000). For each regression model, we calculated standardized selection gradients ( $\beta_\sigma$  and  $\gamma_\sigma$ ) by multiplying original slope values by the population standard deviation for fruit number (Lande and Arnold 1983, Stinchcombe 2005), and provide standard errors in each case.

To test for changes in selection (both linear and nonlinear) associated with diversity and trophic scenarios, we used ANCOVA to compare selection gradients among treatments (Juenger and Bergelson 1998, Strauss et al. 2005). First, we performed an ANCOVA across both levels of diversity and all trophic scenarios (i.e., the entire data set). We modeled relative fitness as dependent upon fruit number, trophic scenario, diversity treatment, all two-way interactions, and the three-way interaction. The three-way interaction was removed from this model because it was not significant, and the two-way trophic scenario  $\times$  diversity interaction was removed because it did not test a hypothesis of interest. Second, following the same procedures described in the previous paragraph for the six separate regression analyses, we added to this ANCOVA model the (fruit number)<sup>2</sup> term and its interactions with diversity and trophic scenario to test for changes in nonlinear selection due to these two factors, again excluding the three-way interaction and the two-way trophic scenario  $\times$  diversity interaction. For the goals of this study, we were specifically interested in fruit number (or [fruit number]<sup>2</sup>)  $\times$  trophic scenario and fruit number (or [fruit number]<sup>2</sup>)  $\times$  diversity interactions. Significant two-way interactions with the linear term (fruit number) indicate a change in strength of directional selection between trophic scenarios or levels of diversity. Significant two-way interactions with the quadratic term ([fruit number]<sup>2</sup>) indicate a difference in the curvature of the fitness function (i.e., change in mode of selection [Conner and Hartl 2004]) between trophic scenarios or levels of diversity.

Contrary to previous findings (Abdala-Roberts et al. 2014), results from these two ANCOVA models showed non-significant fruit number  $\times$  trophic scenario and (fruit number)<sup>2</sup>  $\times$  trophic scenario interactions, indicating that seed predators and parasitoids did not alter the strength or mode of selection on this trait (see *Results*; Appendix: Table A1). However, we found significant fruit number  $\times$  diversity and (fruit number)<sup>2</sup>  $\times$  diversity interactions, revealing that the strength of directional selection as well as the pattern of selection was contingent upon diversity (see *Results*; Appendix: Table

A1). These diversity effects on selection could only occur through altered consumer functional responses because our design eliminated plant–plant interactions and thus effects through resource quantity and quality. In addition, our ecological analyses of diversity effects on consumer functional responses showed effects of diversity on seed predators (response to fruits) but not parasitoids (response to seed predators; see *Results*), based on which we predicted that the effects of diversity on patterns of selection should vary across trophic levels. Accordingly, given that diversity modified the function of plant–seed predator (but not seed predator–parasitoid) interactions, we predicted that diversity would change the strength and/or mode of selection on fruit number under the bi-trophic model but not the tri-trophic model. To evaluate this we tested for an effect of diversity on selection separately within each trophic scenario. Each of these three analyses (one per trophic scenario) was based upon ANCOVA models comparing selection gradients between levels of diversity (separately within each trophic level scenario). First, ANCOVA was used to model relative fitness as dependent upon fruit number, diversity, and the fruit number  $\times$  diversity interaction to test for changes in linear selection due to diversity. Second, and following the same logic used for the previous selection analyses, we added to these models the (fruit number)<sup>2</sup> term and its interactions with diversity to test for changes in nonlinear selection due to diversity. We then qualitatively compared the results of these analyses among the three trophic scenarios.

For the sake of simplicity, we only report results for the interaction terms (and not for main effects) of the ANCOVA models testing for changes in selection between levels of diversity, within each trophic scenario. ANCOVA models based on the entire data set included the effect of plant family, treated as random, to control for using the same plants within each family to compute fitness values for each trophic scenario within each level of diversity. In all cases, data were normally distributed, and this was verified with Shapiro-Wilk tests. All ANCOVA models were performed with PROC MIXED, while regression models were performed in PROC REG, SAS version 9.2 (SAS 2008).

## RESULTS

### *Diversity effects on fruit and insect abundance: initial models*

**Fruit number.**—A total of 6607 fruits (CL = 5049, CH = 1559) were recorded throughout the experiment. We found no effect of diversity on fruit number (monoculture =  $51.14 \pm 6.05$  fruits; polyculture =  $58.70 \pm 5.34$  [raw mean  $\pm$  SE]) (Table 1); accordingly, the genotype sampling effect on fruit number was not statistically different from zero ( $-0.29$ , CI = 2.43). We did find an effect of fruit type, where CL fruits ( $86.03 \pm 5.26$  fruits) were 3.5-fold more abundant than CH fruits ( $24.74 \pm 2.58$ ) across plots (Table 1).

TABLE 1. Summary of results from “initial” models testing for *Ruellia nudiflora* genotype diversity effects on fruit number, seed predator abundance, and parasitoid abundance.

Variable	Source	df	F	P
Fruit number	Diversity	1, 62	1.27	0.26
	Fruit type	1, 62	219.25	<0.0001
Seed predator number	Diversity	1, 55	0.56	0.45
	Fruit type	1, 55	268.55	<0.0001
Parasitoid number	Diversity	1, 54	0.66	0.42
	Fruit type	1, 54	148.13	<0.0001

Notes: Models also accounted for the effects of fruit type (cleistogamous and chasmogamous) and block (significance not tested for). The df column includes numerator and denominator degrees of freedom. Significant effects ( $P < 0.05$ ) are in boldface type.

**Seed predator number.**—A total of 3125 seed predator specimens were recorded, which resulted in  $47.3\% \pm 2.5\%$  (plot mean  $\pm$  SE) of the sampled fruits being attacked by the seed predator. Results from the general linear model indicated no effect of diversity on seed predator abundance (monoculture =  $25.31 \pm 2.56$  seed predators; polyculture =  $26.79 \pm 2.53$  seed predators; Table 1). The sampling effect on seed predator abundance was not statistically different from zero ( $-0.12$ , CI = 0.97).

**Parasitoid number.**—A total of 2178 parasitoid specimens were recorded, resulting in  $76.9\% \pm 2.2\%$  (plot mean  $\pm$  SE) of the seed predator larvae being parasitized. Of the total number of parasitoid specimens recorded, 91% (1974 parasitoids) were *Bracon* sp., followed by Pteromalidae (3%, 65 parasitoids). The remaining 6% (139) were not identified because the parasitoid had exited the fruit prior to fruit collection. Across both parasitoid taxa, we found no effect of diversity on parasitoid abundance (monoculture =  $19.01 \pm 1.95$  parasitoids; polyculture =  $20.11 \pm 1.89$  parasitoids; Table 1). The sampling effect on parasitoid abundance was not statistically different from zero ( $-0.46$ , CI = 0.91).

#### Mechanisms of diversity effects on consumer abundance and interactions

**Seed predator abundance.**—We found no effect of diversity on seed predator abundance. However, we found a significant (fruit number) $^2$   $\times$  diversity interaction (Table 2), indicating that the nonlinear function of fruit–seed predator interactions differed between monocultures and polycultures. Consistent with previous work in this system (Abdala-Roberts and Mooney 2013), we found a saturating (negative) density-dependent recruitment response of the seed predator to fruit abundance in both monocultures (seed predators =  $-0.006 \times [\text{fruit number}]^2 + 0.95 \times \text{fruit number} - 5.33$ ;  $R^2 = 0.80$ ,  $P < 0.0001$ ) and polycultures (seed predators =  $-0.002 \times [\text{fruit number}]^2 + 0.51 \times \text{fruit number} + 3.98$ ;  $R^2 = 0.80$ ,  $P < 0.0001$ ) (Fig. 1A). However, relative to monocultures, seed predator recruitment in polycultures saturated

weakly, with fewer seed predators per fruit at intermediate fruit abundance and more seed predators per fruit at high fruit abundance (Fig. 1A). When expressed in fractional terms, the proportion of attacked fruits in monocultures decreased linearly with increasing fruit number (proportion of attacked fruits =  $-0.0037 \times \text{fruit number} + 0.71$ ;  $R^2 = 0.44$ ,  $P = 0.0001$ ), whereas in polycultures the relationship was nonlinear (proportion of attacked fruits =  $-0.00007 \times [\text{fruit number}]^2 - 0.013 \times \text{fruit number} + 0.98$ ;  $R^2 = 0.70$ ,  $P < 0.0001$ ), because the proportion of attacked fruits decreased more slowly at intermediate fruit numbers and showed no further decrease at high fruit numbers (Fig. 1A, inset). This pattern led to a lower proportion of attacked fruits at intermediate fruit numbers in polycultures relative to monocultures (Fig. 1A, inset).

**Parasitoid abundance.**—After accounting for seed predator number, we found no effect of diversity on parasitoid abundance. Parasitoids exhibited a linear response to seed predator abundance, and this was given by a significant linear term and a non-significant quadratic term for seed predator number (Table 2). We found that seed predator–parasitoid interactions were not altered by diversity, as this linear relationship was similar in both monocultures (parasitoid number =  $0.83 \times \text{seed predator number} - 2.23$ ;  $R^2 = 0.90$ ,  $P < 0.0001$ ) and polycultures (parasitoid number =  $0.85 \times \text{seed predator number} - 2.76$ ;  $R^2 = 0.93$ ,  $P < 0.0001$ ), and remained unchanged across levels of diversity (i.e., non-significant seed predator number  $\times$  diversity term; overall relationship, parasitoid number =  $0.84 \times \text{seed predator number} - 2.48$ ;  $R^2 = 0.91$ ,  $P < 0.0001$ ; Table 2, Fig. 1B); likewise, the (seed predator number) $^2$   $\times$  diversity interaction was also non-significant (Table 2). In addition, the overall relationship between the

TABLE 2. Summary of results from “mechanistic” models testing for effects of *Ruellia nudiflora* genotype diversity on plant–seed predator and seed predator–parasitoid interactions (i.e., diversity-mediated interaction modifications).

Variable	Source	df	F	P
Seed predator (SP) number	diversity ( <i>D</i> )	1, 51	0.05	0.83
	fruit type	1, 51	28.69	<0.0001
	fruit number	1, 51	82.56	<0.0001
	(fruit number) $^2$	1, 51	16.41	0.0002
	fruit number $\times D$	1, 51	2.08	0.15
	(fruit number) $^2 \times D$	1, 51	6.25	0.01
Parasitoid number	diversity ( <i>D</i> )	1, 50	0.08	0.78
	fruit type	1, 50	31.63	<0.0001
	SP abundance	1, 50	165.13	<0.0001
	(SP abundance) $^2$	1, 50	0.39	0.53
	SP abundance $\times D$	1, 50	0.04	0.83
	(SP abundance) $^2 \times D$	1, 50	0.05	0.82

Notes: The df column includes numerator and denominator degrees of freedom. Significant effects ( $P < 0.05$ ) are in boldface type.

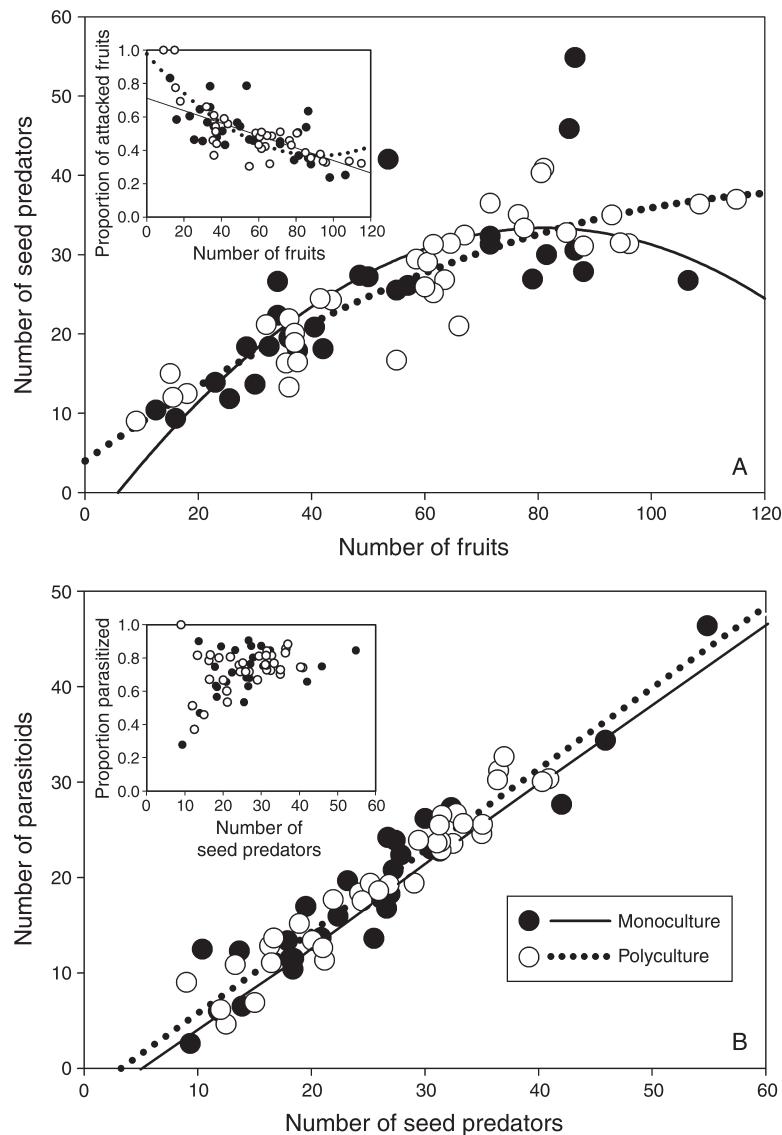


FIG. 1. (A) Relationship between the number of *Ruellia nudiflora* fruits produced and seed predator abundance and (B) between the number of seed predators and parasitoids, shown separately for low and high plant genotype diversity; predicted relationships based upon (A) quadratic or (B) linear regression models are presented for each level of diversity. Insets depict proportional responses between (inset A) the number of fruits and the proportion of attacked fruits, and (inset B) between the number of seed predators and the proportion of parasitized seed predators. The data points represent plot-level least-square means for fruit, seed predator, and parasitoid number based upon a general linear model accounting for fruit type (cleistogamous or chasmogamous).

proportion of parasitized seed predators and seed predator abundance was linear and significantly (albeit weakly) positive (proportion of parasitized seed predators =  $0.004 \times$  seed predator number + 0.63;  $R^2 = 0.07$ ,  $P = 0.04$ ), suggesting positive density-dependent attack by parasitoids (Fig. 1B, inset). Although this fractional response contradicts the previous finding of linear (i.e., constant) recruitment of parasitoids to seed predators, this pattern was nonetheless weak (low  $R^2$ ); based on these facts we conclude that overall (across both levels of diversity) parasitoid attack is weakly density dependent.

#### Evolutionary consequences of plant genotype diversity

Simple regression models performed under each trophic scenario and within each level of diversity showed significant positive directional selection on fruit number for all three trophic scenarios under both high and low diversity (Table 3). However, nonlinear regression models showed that the quadratic term was significant (negative) only for polycultures under the bi-trophic scenario ( $t = 2.62$ ,  $P = 0.02$ ), suggesting stabilizing selection on fruit number (Table 3). The quadratic term was not significant in all the other models ( $t \leq 1.19$ ,  $P \geq 0.25$ ; Table 3).

TABLE 3. Measurements of selection on *Ruellia nudiflora* fruit number at low and high plant genotype diversity, under different “trophic” scenarios (see Notes for this table).

Diversity	Trophic status	$\beta$ (SE)	$\beta_\sigma$ (SE)	$R^2$	$\gamma$ (SE)	$\gamma_\sigma$ (SE)	$R^2$
Monoculture	mono-	0.057 (0.004)**	0.91 (0.064)	0.93	-0.0008 (0.0007)	-0.014 (0.011)	0.92
	bi-	0.067 (0.007)**	1.07 (0.112)	0.88	0.001 (0.001)	0.016 (0.016)	0.88
	tri-	0.057 (0.004)**	0.91 (0.064)	0.96	0.00001 (0.0006)	0.0001 (0.01)	0.96
Polyculture	mono-	0.051 (0.003)**	0.81 (0.048)	0.96	0.00007 (0.005)	0.001 (0.08)	0.88
	bi-	0.052 (0.002)**	0.83 (0.003)	0.88	-0.002 (0.0007)*	-0.031 (0.011)	0.92
	tri-	0.052 (0.003)**	0.83 (0.048)	0.95	-0.0005 (0.0005)	-0.008 (0.008)	0.96

Notes: “Mono-trophic” selection on fruit number assumes no seed predator or parasitoid effects on seed output; “bi-trophic” selection accounts for seed predation but not seed rescue by parasitoids; and “tri-trophic” selection accounts for both seed predator and parasitoid effects on seed number.  $\beta$ 's are the directional selection gradients on fruit number when only the linear fruit number term was included in the model, whereas  $\gamma$ 's are the nonlinear selection gradients when both the linear and quadratic terms were included in the model (Scheiner et al. 2000). The quadratic term was significant only for the polyculture treatment under the bi-trophic scenario. Both unstandardized ( $\beta$ ,  $\gamma$ ) and standardized ( $\beta_\sigma$ ,  $\gamma_\sigma$ ) selection gradients and standard errors (SE) are reported, as well as model  $R^2$  values.

\*  $P < 0.05$ ; \*\*  $P < 0.001$

Our ANCOVA test for changes in selection (both linear and nonlinear) associated with diversity and trophic scenario showed nonsignificant fruit number  $\times$  trophic scenario or (fruit number) $^2$   $\times$  trophic scenario interactions, indicating that seed predators and parasitoids did not alter the strength or mode of selection on this trait (Appendix: Table A1). However, we found significant fruit number  $\times$  diversity and (fruit number) $^2$   $\times$  diversity interactions, revealing that patterns of selection were contingent upon diversity (Appendix: Table A1). Subsequent ANCOVA models conducted within each trophic scenario showed that diversity did not change the strength of directional selection (diversity  $\times$  fruit number term,  $F_{1,24} = 1.30$ ,  $P = 0.26$ ) or mode of selection (diversity  $\times$  [fruit number] $^2$ ,  $F_{1,24} = 1.07$ ,  $P = 0.31$ ) under the mono-trophic scenario (Fig. 2A). In contrast, under the bi-trophic scenario we found a tendency for weaker directional selection at high relative to low diversity (diversity  $\times$  fruit number,  $F_{1,22} = 2.83$ ,  $P = 0.10$ ), as well as a significant change from directional selection at low diversity to nonlinear selection at high diversity (diversity  $\times$  [fruit number] $^2$ :  $F_{1,22} = 5.36$ ,  $P = 0.03$ ) (Fig. 2B). Because selection changed between levels of diversity under the bi-trophic scenario (and not under the mono-trophic scenario), this suggests that seed predators are responsible for the diversity-mediated change in selection. More specifically, this shows that the change in the seed predator's functional response at high diversity (Fig. 1A) altered the pattern of selection on fruit number. Interestingly, under the tri-trophic scenario we again found no evidence of a change in the strength of directional selection (diversity  $\times$  fruit number,  $F_{1,24} = 1.71$ ,  $P = 0.20$ ) or mode of selection (diversity  $\times$  [fruit number] $^2$ ,  $F_{1,24} = 0.85$ ,  $P = 0.36$ ) (Fig. 2C), suggesting that parasitoids eliminated the linkage between plant diversity and seed predator selection.

## DISCUSSION

Our study shows that plant intraspecific diversity mediated the function of plant–seed predator, but not seed predator–parasitoid interactions. Such diversity

effect on plant–seed predator interactions in turn fed back to change natural selection on fruit number, with seed predators imposing nonlinear selection at high diversity, but only in the absence of top-down effects by parasitoids. This latter finding indicates that parasitoids eliminated the effect of diversity on seed predator selection. Importantly, such effects were not driven by differences in resource quantity (i.e., fruit production) or quality between monocultures and polycultures; below- and aboveground interactions among plants were prevented, and there were no detectable genotype sampling effects. Accordingly, the observed effects of diversity occurred through resource heterogeneity effects on consumer foraging behaviors. In addition, these findings come within the context of genetic variation in a single source population, making our evaluation of diversity effects and evolutionary dynamics realistic with respect to natural processes (Tack et al. 2012). Overall, these findings provide novel evidence for the mechanistic basis of feedbacks between plant diversity and herbivore selection, as well as the role of carnivores in mediating such effects.

### Plant diversity causes interaction modifications

Although evidence is mounting for the effects of plant intra- and interspecific diversity on higher trophic levels (Bailey et al. 2009, Haddad et al. 2009, Scherber et al. 2010), most studies have not tested whether such effects operate via density-mediated or interaction modification effects (but see Moreira et al. 2012, Moreira and Mooney 2013). A few studies have reported density-mediated effects where increased producer biomass at high diversity (presumably via resource niche partitioning or facilitation) results in greater herbivore and predator abundance and diversity (Koricheva et al. 2000, Crutsinger et al. 2006, Johnson et al. 2006, Genung et al. 2010, Cook-Patton et al. 2011, McArt et al. 2012; but see Crawford and Rudgers 2013). However, most research has failed to explicitly address the mechanisms by which plant diversity influences consumer interactions (for exceptions see Utsumi et al.

2011, Moreira et al. 2012, McArt and Thaler 2013, Moreira and Mooney 2013).

We found that plant intraspecific diversity altered the function of fruit–seed predator interactions (Fig. 1A), and this mechanism operated in the absence of density-mediated effects via increased plant biomass (i.e., diversity had no effect on fruit number) (e.g., see Crawford and Rudgers 2013). It is unsurprising that diversity had no influence on fruit production, as we prevented both below- and aboveground plant interactions, which frequently lead to increased plant biomass via sampling and complementarity effects. Such effects might have otherwise caused greater resource and consumer abundance (i.e., density-mediated effects [Crutsinger et al. 2006, Cook-Patton et al. 2011, McArt et al. 2012]). Our experimental approach thus isolated the effects of resource heterogeneity on consumer foraging behavior and shows that plant diversity effects on higher trophic levels can operate through interaction modifications. We recognize, however, that density-mediated effects of diversity are widespread and can have large effects on consumers. Ultimately, plant diversity effects likely operate through some combination of density-mediated and trait-mediated resource heterogeneity effects, and future experiments should formally compare the strength of these two mechanisms.

Previous work by Moreira et al. (2012) and Moreira and Mooney (2013) tested for plant diversity-mediated interaction modifications, but only the former found evidence for altered interactions (between aphids and their tending ants; see also Haddad et al. 2009). In our study, we found that seed predator recruitment to fruits resembled a Type II functional response (sensu Holling 1959) at both high and low diversity. However, the shape of this Type II response depended on plant diversity; relative to monoculture, the rate of herbivore recruitment in polyculture was lower at intermediate fruit abundance and greater at high fruit abundance (Fig. 1A). Such a pattern (i.e., initially low and subsequently higher recruitment) resulted in no overall change in herbivore abundance between levels of diversity. This finding illustrates the importance of using functional responses to describe changes in the strength of species interactions, and thus for understanding and predicting the consequences of interaction modifications caused by habitat or resource heterogeneity (Alexander et al. 2013). One mechanism that could explain the observed effect of diversity on seed predator functional responses is the influence of within-patch trait heterogeneity on herbivore foraging behavior and host detection mechanisms (Bommarco and Banks 2003, Agrawal et al. 2006, Hämbäck et al. 2009). Although speculative, it is possible that greater variability in flower volatiles, inflorescence height, or plant architecture at high diversity played an important role in altering moth search and oviposition patterns (Cuautle and Parra-Tabla 2014; see also Langellotto and Denno 2004, Ninkovic et al. 2011).

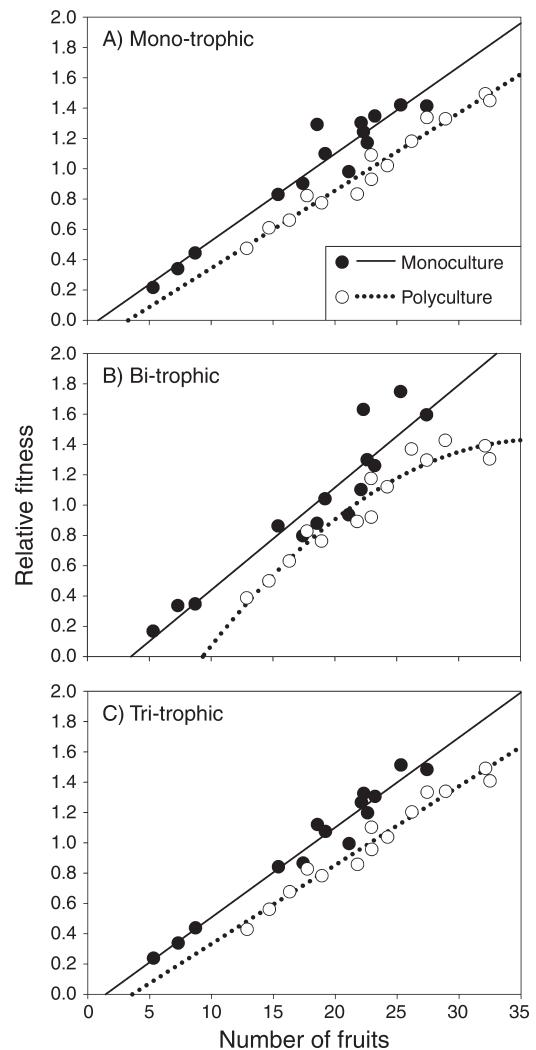


FIG. 2. Patterns of natural selection on *Ruellia nudiflora* fruit number at high and low plant genotype diversity under three trophic scenarios: (A, “mono-trophic”) in the absence of seed predator and parasitoid effects on plant fitness, (B, “bi-trophic”) accounting for seed predator effects but not parasitoid seed rescue, and (C, “tri-trophic”) accounting for both seed predator and parasitoid effects on plant fitness. Relative fitness values were calculated within each trophic scenario and level of diversity. Selection on fruit number did not differ between levels of diversity under the mono- and tri-trophic selection gradients (positive, directional in both cases), but did differ under the bi-trophic scenario where it changed from directional selection at low diversity to stabilizing selection at high diversity. The comparison of slopes among trophic scenarios was the subject of another experiment (L. Abdala-Roberts et al. *unpublished manuscript*). Data points represent plant family means.

Contrary to plant–seed predator interactions, diversity did not influence seed predator–parasitoid interactions, which suggests that plant traits mediating diversity effects on seed predators do not have parallel effects on parasitoids. In addition, this result is perhaps surprising given mounting evidence for the use of plant cues by predators and parasitoids in foraging for



PLATE 1. Parasitoid wasp on a chasmogamous fruit of *Ruellia nudiflora*, at a site located near Merida, Yucatan (Mexico). Photo credit: Luis Salinas-Peba.

herbivores (Heil 2008), and heterogeneity in such cues mediating plant diversity effects on predator behavior (Ninkovic et al. 2011). In accordance with our findings, however, previous work has shown that plant genetic effects are stronger on herbivores than on carnivores (e.g., Johnson and Agrawal 2005, Bailey et al. 2009, Schädler et al. 2010, Scherber et al. 2010; but see Johnson 2008), supporting the notion that herbivores respond more strongly to plant trait variation than do predators (Johnson 2008). In addition, some groups of predators (e.g., parasitoids in our system) exhibit highly efficient foraging strategies and may be able to overcome or be less influenced by the effects of habitat heterogeneity produced by plant genotype variation (Abdala-Roberts and Mooney 2013).

#### *Evolutionary consequences of plant diversity*

Interaction modifications are increasingly recognized as an important source of evolutionary change (Miller and Travis 1996, Inouye and Stinchcombe 2001, Strauss and Irwin 2004, Mooney and Singer 2012). Accordingly, our findings suggest that they may provide a useful framework for understanding and predicting the evolutionary consequences of plant diversity effects on consumers. However, to date, very few studies have explicitly tested for plant diversity-mediated interaction modifications (e.g., Moreira et al. 2012, Moreira and Mooney 2013) and none have tested the evolutionary consequences of such altered interactions (but see

Parker et al. 2010). Whereas Moreira et al. (2012) and Moreira and Mooney (2013) tested for effects of diversity on the rate of recruitment of predatory ants, only the former found evidence of stronger top-down effects of ants on plant growth at high diversity. However, this study did not evaluate the evolutionary consequences of altered top-down effects (Moreira et al. 2012). In addition, Johnson et al. (2006) found that plant genotype diversity influenced arthropod abundance, which in turn had an impact on plant fitness. Nonetheless, this study did not test for interaction modifications or changes in selection on plant traits. To date, the only study that has addressed the potential for eco-evolutionary feedbacks between plant diversity and trophic interactions was conducted by Parker et al. (2010), who found that plant genetic diversity influenced herbivory by voles and deer, which in turn led to altered patterns of selection on plant traits.

Our study provides direct evidence that plant diversity, in shaping plant–herbivore interactions, can lead to changes in herbivore selection on plants (Agrawal et al. 2006). Relative to monocultures, seed predator recruitment in polycultures was weaker at intermediate fruit abundance and greater at high fruit abundance, resulting in a change from directional selection for increased fruit number at low diversity, to nonlinear selection at high diversity. Although this difference in seed predator selection between low and high diversity was not strong, it nonetheless suggests that genotypes

with intermediate fruit number were selectively favored in polycultures (i.e., stabilizing selection; Fig. 2B). Interestingly, this change in mode of selection at high diversity became apparent only under the bi-trophic scenario. Because this pattern was not observed in the absence of seed predator effects (mono-trophic scenario), this indicates that seed predators were responsible for the change in selection across levels of diversity. Moreover, the fact that diversity failed to alter selection under the tri-trophic scenario suggests that parasitoids prevented a feedback between diversity and seed predator selection. This latter finding is biologically relevant, because previous work has shown that parasitism levels vary greatly among *R. nudiflora* populations (0–65% [Abdala-Roberts et al. 2010]) which may lead to differences in the strength of these feedbacks (this study representing an extreme case where high parasitism prevents a feedback). Because spatial variation in consumer species composition and the intensity of interactions is widespread across systems (Thompson 2005, Gripenberg and Roslin 2007), our findings suggest that geographic variation in plant diversity–consumer feedbacks is common and may be contingent upon predator or parasitoid top-down effects. It is also interesting to note that parasitoid effects were only present at high plant diversity, suggesting that trophic complexity interacts with diversity within trophic levels to determine patterns of selection on plant traits (see Parker et al. 2010).

While this study focused on the patterns of selection for increased fruit number in the first year of growth, it is likely that any such evolutionary response would trade off with reproduction in subsequent years. Accordingly, in this study we found threefold variation among families in fruit production during the first growing season, supporting the idea that trade-offs between present and future reproduction maintain genetic variation in reproductive display size. Previous work has also shown a negative genetic correlation between chasmogamous and cleistogamous reproduction, suggesting that a reproductive trade-off is present in this aspect of *R. nudiflora*'s reproductive strategy as well (Abdala-Roberts et al. 2014). Therefore, it is possible that the observed selective effects by seed predators and parasitoids on fruit number may vary across growing seasons (via present–future reproductive trade-offs), as well as differentially influence each reproductive function (via chasmogamy–cleistogamy trade-offs). Having said this, however, patterns of selection during the first year of growth recorded in the present study probably have a large influence on lifetime fitness for short-lived perennials such as *R. nudiflora*.

#### *Concluding remarks*

Most previous work lacks an explicit consideration of the mechanisms by which plant diversity influences consumers, and the evolutionary consequences of such effects remain largely untested. Our study emphasizes

the value of using functional responses to depict interaction modifications. Accordingly, we show that linking consumer functional responses to patterns of selection allows us to understand and predict the evolutionary outcomes of complex (multi-trophic) interactions. Our findings also underscore that this approach can be used to evaluate the ecological and evolutionary consequences of plant diversity or any other source of variation in plant bottom-up effects on higher trophic levels. Finally, we suggest that there is widespread potential for eco-evolutionary feedbacks between plant bottom-up forcing and consumer top-down effects. If such feedbacks involve plant intraspecific diversity, then there is the possibility for altered patterns (strength and mode) of selection among plant patches with varying diversity. These dynamics may shape the amount and spatial distribution of genetic variation within populations, which will then influence consumers.

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#### LITERATURE CITED

- Abdala-Roberts, L., D. Marrufo-Zapata, G. Arceo-Gómez, and V. Parra-Tabla. 2014. Pollen limitation, fruit abortion, and autonomous selfing in three populations of the perennial herb *Ruellia nudiflora*. *Plant Species Biology* 29:25–33.
- Abdala-Roberts, L., and K. A. Mooney. 2013. Environmental and plant genetic effects on tri-trophic interactions. *Oikos* 152:1157–1166.
- Abdala-Roberts, L., V. Parra-Tabla, D. Campbell, and K. A. Mooney. 2014. Top-down and bottom-up forces shape herbivore selection on plant traits. *Journal of Ecology*, *in press*.
- Abdala-Roberts, L., V. Parra-Tabla, L. Salinas-Peba, C. Diaz-Castelazo, and H. Delfin-Gonzalez. 2010. Spatial variation in the strength of a trophic cascade involving *Ruellia nudiflora* (Acanthaceae), an insect seed predator and associated parasitoid fauna in Mexico. *Biotropica* 42:180–187.
- Abrams, P. A. 1995. Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. *American Naturalist* 146: 112–134.
- Agrawal, A. A., J. Lau, and P. A. Hämbäck. 2006. Community heterogeneity and the evolution of interactions between plants and insect herbivores. *Quarterly Review of Biology* 81:349–376.
- Alexander, M. E., J. T. A. Dick, and N. E. O'Connor. 2013. Trait-mediated indirect interactions in a marine intertidal system as quantified by functional responses. *Oikos* 122: 1521–1531.
- Andow, D. A. 1991. Vegetational diversity and arthropod population response. *Annual Review of Entomology* 36:561–586.

- Bailey, J., et al. 2009. From genes to ecosystems: a synthesis of the effects of plant genetic factors across levels of organization. *Philosophical Transactions of the Royal Society* 364: 1607–1616.
- Bolnick, D. I., P. Amarasekare, M. S. Araújo, R. Bürger, J. M. Levine, M. Novak, V. H. W. Rudolf, S. J. Schreiber, M. C. Urban, and D. A. Vasseur. 2011. Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution* 26:183–192.
- Bommarco, R., and J. E. Banks. 2003. Scale as a modifier in vegetation diversity experiments: effects on herbivores and predators. *Oikos* 102:440–448.
- Cardinale, B. J., A. R. Ives, and P. Inchausti. 2004. Effects of species diversity on the primary productivity of ecosystems: extending our spatial and temporal scales of inference. *Oikos* 104:437–450.
- Cardinale, B., K. L. Matulich, D. U. Hooper, J. E. Byrnes, E. Duffy, L. Gamfeldt, P. Balvanera, M. I. O'Connor, and A. González. 2011. The functional role of producer diversity in ecosystems. *American Journal of Botany* 98:572–592.
- Castagneyrol, B., B. Giffard, C. Pére, and H. Jactel. 2013. Plant apparency; an overlooked driver of associational resistance to insect herbivory. *Journal of Ecology* 101:418–429.
- Conner, J. K., and D. L. Hartl. 2004. A primer of ecological genetics. Sinauer, Sunderland, Massachusetts, USA.
- Cook-Patton, S. C., S. H. McArt, A. L. Parachnowitsch, J. S. Thaler, and A. A. Agrawal. 2011. A direct comparison of the consequences of plant genotypic and species diversity on communities and ecosystem function. *Ecology* 92:915–923.
- Cottingham, K. L., B. L. Brown, and J. T. Lennon. 2001. Biodiversity may regulate the temporal variability of ecological systems. *Ecology Letters* 4:72–85.
- Crawford, K. M., and J. A. Rudgers. 2013. Genetic diversity within a dominant plant outweighs plant species diversity in structuring an arthropod community. *Ecology* 94:1025–1035.
- Crutsinger, G. M., M. D. Collins, J. A. Fordyce, Z. Gompert, C. C. Nice, and N. J. Sanders. 2006. Plant genotypic diversity predicts community structure and governs an ecosystem process. *Science* 313:966–968.
- Crutsinger, G. M., W. N. Reynolds, A. T. Classen, and N. J. Sanders. 2008. Disparate effects of plant genotypic diversity on foliage and litter arthropod communities. *Oecologia* 158: 65–75.
- Cuautle, M., and V. Parra-Tabla. 2014. Describing a multi-trophic plant-herbivore-parasitoid system at four spatial scales. *Acta Oecologica* 55:8–14.
- Fairbairn, D. J., and R. F. Preziosi. 1994. Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider, *Aquarius remigis*. *American Naturalist* 144: 101–118.
- Genung, M. A., et al. 2010. Non-additive effects of genotypic diversity increase floral abundance and abundance of floral visitors. *PloS One* 5:e8711.
- Genung, M. A., J. A. Scheitzer, F. Ubeda, B. M. Fitzpatrick, C. C. Pregitzer, E. Felker-Quinn, and J. K. Bailey. 2011. Genetic variation and community change: selection, evolution, and feedbacks. *Functional Ecology* 25:408–419.
- Gripenberg, S., and T. Roslin. 2007. Up or down in space? Uniting the bottom-up versus top-down paradigm and spatial ecology. *Oikos* 116:181–188.
- Haddad, N., G. M. Crutsinger, K. Gross, J. Haarstad, J. M. H. Knops, and D. Tilman. 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. *Ecology Letters* 12:1029–1039.
- Hämbäck, P. A., M. Björkman, B. Rämert, and R. J. Hopkins. 2009. Scale-dependent responses in cabbage herbivores affect attack rates in spatially heterogeneous systems. *Basic and Applied Ecology* 10:228–236.
- Heil, M. 2008. Indirect defence via tritrophic interactions. *New Phytologist* 178:41–61.
- Herrera, C. M. 2000. Measuring the effects of pollinators and herbivores: evidence for non-additivity in a perennial herb. *Ecology* 81:2170–2176.
- Holling, C. S. 1959. Some characteristics of simple types of predation and parasitism. *Canadian Entomologist* 91:385–398.
- Hooper, D. U., et al. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. *Ecological Monographs* 75:3–35.
- Houston, M. A. 1997. Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia* 110:449–460.
- Hughes, R. A., B. T. Inouye, M. T. Johnson, N. Underwood, and M. Vellend. 2008. Ecological consequences of genetic diversity. *Ecology Letters* 11:609–623.
- Inouye, B., and J. R. Stinchcombe. 2001. Relationships between ecological interaction modifications and diffuse coevolution: similarities, differences, and causal links. *Oikos* 95:353–360.
- Johnson, K. H., K. A. Vogt, H. J. Clark, O. J. Schmitz, and D. J. Vogt. 1996. Biodiversity and the productivity and stability of ecosystems. *Trends in Ecology and Evolution* 11: 372–377.
- Johnson, M. T. J. 2008. Bottom-up effects of plant genotype on aphids, ants, and predators. *Ecology* 89:145–154.
- Johnson, M. T. J., and A. A. Agrawal. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology* 86:874–885.
- Johnson, M. T. J., M. J. Lajeunesse, and A. A. Agrawal. 2006. Additive and interactive effects of plant genotypic diversity on arthropod communities and plant fitness. *Ecology Letters* 9:23–34.
- Juenger, T., and J. Bergelson. 1998. Pairwise versus diffuse natural selection in the multiple herbivores of scarlet gilia. *Evolution* 52:1583–1592.
- Koricheva, J., C. P. H. Mulder, B. Schmid, J. Joshi, and K. Huss-Danell. 2000. Numerical responses of different trophic groups of invertebrates to manipulations of plant diversity in grasslands. *Oecologia* 125:271–282.
- Lande, R., and R. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Langellotto, G. A., and R. F. Denno. 2004. Responses of invertebrate natural enemies to complex-structured habitats: a meta-analytical synthesis. *Oecologia* 139:1–10.
- Long, R. W. 1977. Artificial induction of obligate cleistogamy in species-hybrids in *Ruellia* (Acanthaceae). *Bulletin of the Torrey Botanical Club* 104:53–56.
- Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. *Nature* 412:72–76.
- McArt, S. H., S. C. Cook-Patton, and J. S. Thaler. 2012. Relationships between arthropod richness, evenness, and diversity are altered by complementarity among plant genotypes. *Oecologia* 168:1013–1021.
- McArt, S. H., and J. S. Thaler. 2013. Plant genotypic diversity reduces the rate of consumer resource utilization. *Proceedings of the Royal Society B* 280:20130639.
- Miller, T. E., and J. Travis. 1996. The evolutionary role of indirect effects in communities. *Ecology* 77:1329–1335.
- Mooney, K. A., and A. A. Agrawal. 2008. Plant genotype shapes ant-aphid interactions: implications for community structure and indirect plant defense. *American Naturalist* 171:E195–E205.
- Mooney, K. A., and M. S. Singer. 2012. Plant effects on herbivore-enemy interactions in natural systems. Pages 107–130 in T. Ohgushi, O. Schmitz, and R. D. Holt, editors. *Trait-mediated indirect interactions: ecological and evolutionary perspectives*. Cambridge University Press, New York, New York, USA.

- Moreira, X., and K. A. Mooney. 2013. Influence of plant genetic diversity on interactions between higher trophic levels. *Biology Letters* 9:20130133.
- Moreira, X., K. A. Mooney, R. Zas, and L. Sampedro. 2012. Bottom-up effects of host-plant species diversity and top-down effects of ants interactively increase plant performance. *Proceedings of the Royal Society B* 279:4464–4472.
- Munguía-Rosas, M., L. Abdala-Roberts, and V. Parra-Tabla. 2013. Effects of pollen load, parasitoids and the environment on pre-dispersal seed predation in the cleistogamous *Ruellia nudiflora*. *Oecologia* 173:871–880.
- Munguía-Rosas, M. A., V. Parra-Tabla, J. Ollerton, and J. C. Cervera. 2012. Environmental control of reproductive phenology and the effect of pollen supplementation on resource allocation in the cleistogamous weed *Ruellia nudiflora* (Acanthaceae). *Annals of Botany* 109:343–350.
- Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1994. Declining biodiversity can alter the performance of ecosystems. *Nature* 368:734–737.
- Ninkovic, V., S. A. Abassi, E. Ahmed, R. Glinwood, and J. Pettersson. 2011. Effect of within-species plant genotype mixing on habitat preference of a polyphagous insect predator. *Oecologia* 166:391–400.
- Ortegón-Campos, I., L. Abdala-Roberts, V. Parra-Tabla, J. C. Cervera, D. Marrufo-Zapata, and C. M. Herrera. 2012. Influence of multiple factors on plant local adaptation: soil type and folivore effects in *Ruellia nudiflora* (Acanthaceae). *Evolutionary Ecology*. <http://dx.doi.org/10.1007/s10682-011-9507-5>
- Östergård, H., P. Hämäläinen, and J. Ehrlén. 2007. Pre-dispersal seed predation: the role of fruit abortion and selective oviposition. *Ecology* 88:2959–2965.
- Parker, J. D., J. P. Salminen, and A. A. Agrawal. 2010. Herbivory enhances positive effects of plant genotypic diversity. *Ecology Letters* 13:553–563.
- Pilson, D. 2000. Herbivory and natural selection on flowering phenology in wild sunflower, *Helianthus annuus*. *Oecologia* 122:72–82.
- Plath, M., S. Dorn, J. Riedel, H. Barrios, and K. Mody. 2012. Associational resistance and associational susceptibility: specialist herbivores show contrasting responses to tree stand diversification. *Oecologia* 169:477–487.
- Post, D. M., and E. P. Palkovacs. 2009. Eco-evolutionary feedbacks in community and ecosystem ecology: interactions between the ecological theatre and the evolutionary play. *Philosophical Transactions of the Royal Society B* 364:1629–1640.
- Raffaelli, D., W. H. van der Putten, L. Persson, D. A. Wardle, O. L. Petchey, J. Koricheva, M. van der Heijden, J. Mikola, and T. Kennedy. 2002. Multi-trophic dynamics and ecosystem processes. Pages 147–154 in M. Loreau, S. Naeem, and P. Inchausti, editors. *Biodiversity and ecosystem functioning: synthesis and perspectives*. Oxford University Press, Oxford, UK.
- Rausher, M. 1992. The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* 46:616–626.
- SAS. 2008. PROC MIXED, version 9.2. SAS Institute, Cary, North Carolina, USA.
- Schädler, M., R. Brandl, and A. Kempel. 2010. Host plant genotype determines bottom-up effects in an aphid-parasitoid-predator system. *Entomologia Experimentalis et Applicata* 135:162–169.
- Scheiner, S. M., R. J. Mitchell, and H. S. Callahan. 2000. Using path analysis to measure natural selection. *Journal of Evolutionary Biology* 13:423–433.
- Scherber, C., et al. 2010. Bottom-up effects of plant diversity on multitrophic interactions in a biodiversity experiment. *Nature* 468:553–556.
- Siemann, E. 1998. Experimental tests of effects of plant productivity and diversity on grassland arthropod diversity. *Ecology* 79:2057–2070.
- Singer, M. S., T. E. Farkas, C. M. Skorik, and K. A. Mooney. 2012. Tritrophic interactions at a community level: effects of host plant species quality on bird predation of caterpillars. *American Naturalist* 179:363–374.
- Stinchcombe, J. R. 2005. Measuring natural selection on proportional traits: comparisons of three types of selection estimates for resistance and susceptibility to herbivore damage. *Evolutionary Ecology* 19:363–373.
- Stinchcombe, J. R., M. T. Rutter, D. S. Burdick, P. Tiffin, M. D. Rausher, and R. Mauricio. 2002. Testing for environmentally induced bias in phenotypic estimates of natural selection: theory and practice. *American Naturalist* 160:511–523.
- Strauss, S. Y., and R. E. Irwin. 2004. Ecological and evolutionary consequences of multispecies plant-animal interactions. *Annual Review of Ecology, Evolution and Systematics* 35:435–466.
- Strauss, S. Y., H. Sahli, and J. K. Conner. 2005. Toward a more trait-centered approach to diffuse (co)evolution. *New Phytologist* 165:81–90.
- Tack, A. J. M., T. J. Johnson, and T. Roslin. 2012. Sizing up community genetics: it's a matter of scale. *Oikos* 121:481–488.
- Thompson, J. N. 2005. *The geographic mosaic of coevolution*. University of Chicago Press, Chicago, Illinois, USA.
- Tilman, D., J. Knops, D. Wedin, and P. Reich. 2002. Plant diversity and composition: effects on productivity and nutrient dynamics of experimental grasslands. Pages 21–35 in M. Loreau, S. Naeem, and P. Inchausti, editors. *Biodiversity and ecosystem functioning: synthesis and perspectives*. Oxford University Press, Oxford, UK.
- Tilman, D., C. Lehman, and K. Thompson. 1997. Plant diversity and ecosystem productivity: theoretical considerations. *Proceedings of the National Academy of Sciences USA* 1857–1861.
- Tilman, D., P. B. Reich, and J. Knops. 2006. Biodiversity and ecosystem stability in a decade-long grassland experiment. *Nature* 44:629–632.
- Tilman, D., D. Wedin, and J. Knops. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379:718–720.
- Utsumi, S., Y. Ando, T. P. Craig, and T. Ohgushi. 2011. Plant genotypic diversity increases population size of an herbivorous insect. *Proceedings of the Royal Society B* 22:3108–3115.
- Wootton, J. T. 1994. The nature and consequences of indirect effects in ecological communities. *Annual Review of Ecology and Systematics* 25:443–466.

## SUPPLEMENTAL MATERIAL

## Appendix

Results from statistical models testing for changes in selection on *Ruellia nudiflora* fruit number across trophic scenarios and levels of plant genotype diversity ([Ecological Archives E095-249-A1](#)).