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Environmental and plant genetic effects on tri-trophic interactions

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The effects of plant genotype and environmental factors on tri-trophic interactions have usually been investigated separately, limiting our ability to compare the relative strength of these effects as well as their potential to interactively shape arthropod communities. We studied the interactions among the herb *Ruellia nudiflora*, a seed predator, and its parasitoids using 14 maternal plant families grown in a common garden. By fertilizing half of the plants of each family and subsequently recording fruit number, seed predator number, and parasitoid number per plant, we sought to compare the strength of plant genetic effects with those of soil fertility, and determine if these factors interactively shape tri-trophic interactions. Furthermore, we evaluated if these bottom–up factors influenced higher trophic levels through changes in abundance across trophic levels (density-mediated) or changes in the function of species interactions (trait-mediated). Plant genetic effects on seed predators and parasitoids were stronger than fertilization effects. Moreover, we did not find plant genetic variation for fertilization effects on fruit, seed predator, or parasitoid abundance, showing that each factor acted independently on plant resources and higher trophic levels. Both bottom–up forces were transmitted via density-mediated effects where increased fruit number from fertilization and plant genetic effects increased seed predator and parasitoid abundance; however, seed predator attack was density-dependent, while parasitoid attack was density-independent. Importantly, there was evidence (marginally significant in one case) that fertilization modified the function of plant-seed predator and seed predator–parasitoid interactions by increasing the number of seed predators per fruit and decreasing the number of parasitoids per seed predator, respectively. These findings show that plant genetic and soil fertility effects cascaded up this simple food chain, that plant genetic effects were stronger across all trophic levels, and that these effects were transmitted independently and through contrasting mechanisms.

Multi-trophic interactions and arthropod community structure can change substantially among genotypes within a single plant species (Dungey et al. 2000, Wimp and Whitham 2001, Johnson 2008, Mooney and Agrawal 2008), with plant genotype effects in some cases being similar in magnitude to plant species effects (Mooney et al. 2010, Singer et al. 2012). Previous studies have shown that plant genotypes can mediate herbivore–herbivore (McGuire and Johnson 2006), herbivore–carnivore (Fritz 1995, Hare 2002, Mooney and Singer 2012), as well as mutualistic (Mooney and Agrawal 2008) interactions. Accordingly, examining the effects of plant genotypes on ecological interactions has served to elucidate the mechanisms by which these effects scale-up to influence entire arthropod communities (Johnson 2008, Mooney and Singer 2012). A recent focus has been to evaluate how plant genotypes are influenced by biotic and abiotic factors, documenting how environmental conditions and plant genetics interactively control plant traits of importance for tri-trophic interactions (Poelman et al. 2009, Abdala-Roberts et al. 2012). Although this approach provides a useful mechanistic framework for studying how bottom–up forces concurrently shape arthropod communities, few studies have examined the combined effects of plant genotype and the abiotic environment under a multi-trophic setting (Johnson and Agrawal 2005, Abdala-Roberts et al. 2012).

Multiple studies have shown that bottom–up effects of soil fertility can influence plant–herbivore interactions through changes in plant tissue quality (e.g. defenses; Orians et al. 2003, Sampedro et al. 2011) or quantity (i.e. biomass production; Stiling and Rossi 1997), which in turn influence carnivores due to changes in herbivore abundance or quality (Williams 1999, Chen et al. 2010). In addition, studies have also found evidence of plant genetic variation for soil fertilization effects on plant–herbivore interactions (Horner and Abrahamson 1992, Orians and Fritz 1996, Rowntree et al. 2010), likely due to plant genotypes responding differentially to fertilization because of underlying growth-defense tradeoffs (Coley et al. 1985; reviewed by Stamp 2003, Sampedro et al. 2011). Nonetheless, few studies have simultaneously evaluated the effects plant genetics and soil fertility exert on the third trophic level, thus limiting our understanding of the relative importance and potential for interaction of bottom–up forces shaping complex interactions and, ultimately, arthropod community structure.

Although there are different mechanisms by which plant genotype and soil fertility can indirectly influence species at higher trophic levels, these are frequently not addressed and their relative importance is uncertain. First, changes in plant traits due to plant genotype and fertilization can result in density-mediated effects ('interaction chains', sensu Wootton 1994). Here, plant trait variation directly influences the density of one arthropod species (e.g. herbivore), which in turn indirectly influences the density of a second species (e.g. carnivore), but the function describing their pairwise (e.g. herbivore–carnivore) interaction – which can be linear or non-linear – remains unchanged (Mooney and Agrawal 2008, Singer et al. 2012). Secondly, changes in plant traits may modify the function describing this pairwise interaction through changes in traits of the interacting species ('interaction modification' sensu Wootton 1994). Here, fertilization and plant genetic effects on plant traits may influence pairwise interactions through effects on the traits or behaviors of one or both interacting species. Identifying which of these two pathways of plant indirect effects is acting on higher-trophic levels is important because it provides a mechanistic framework to predict the ecological and evolutionary consequences of plant mediation of higher trophic-level interactions (Abrams 1995, Strauss et al. 2005, Mooney and Singer 2012).

Ruellia nudiflora (Acanthaceae) is a common herb found in southern Mexico that suffers from high rates of seed predation by larvae of a noctuid moth (Abdala-Roberts et al. 2010). In turn, this herbivore is fed upon by up to eight species of parasitoids with different life histories. Here we focus on *R. nudiflora* fruit production as a key resource governing species interactions in this food chain and address the following questions: (a) do soil fertility and plant genetic effects, in controlling variation for a basal resource (fruits) influence seed predator abundance, and parasitoid abundance? (b) do these bottom–up forces have effects of similar magnitude and do they operate independently or interactively (i.e. is there plant genetic variation for fertility effects on trophic interactions)? And (c) do these effects occur through density-mediated or interaction modification effects? This work is novel in that it compares the influence of two sources of variation for plant-based resources on interactions among species at higher trophic levels, and examines the mechanisms by which such effects take place. In doing so, our findings advance the understanding of how bottom–up effects influence food chains associated with plant canopies.

Methods

Study species and natural history

Ruellia nudiflora is a self-compatible, perennial herb distributed from Texas to southeast Mexico. It is abundant in the state of Yucatan (Mexico) and grows under a wide range of climatic and soil conditions (Ortegon-Campos et al. 2012). It has a mixed mating system and produces chasmogamous (CH) flowers which open and are visited by pollinators, as well as cleistogamous (CL) flowers which have a reduced corolla, do not open and obligately self-pollinate; both flower types have a longevity of one day. The peak of flower production is typically during July or August, although flowering may extend up to December in some cases. Both types of fruit are dry and dehiscent.

Fruits from both CH and CL flowers are attacked by larvae of a single, as-yet unidentified, species of noctuid moth (Lepidoptera: Noctuidae) which feed on seeds prior to fruit dehiscence. Adult female moths oviposit on recently pollinated flowers and, unless parasitized, a single larva grows inside each developing fruit and usually consumes all the seeds (Abdala-Roberts et al. 2010). There is a negative relationship between fruiting synchrony (at the population level) and the proportion of fruits attacked (V. Parra-Tabla unpubl.), presumably because synchronous fruiting results in herbivore satiation (reviewed by Elzinga et al. 2007). Herbivore eggs or larvae are attacked by up to seven, as-yet unidentified species of parasitic wasps (Hymenoptera) and one fly (Diptera), namely: one wasp species of Ichneumonidae, four of Braconidae (two species of *Bracon*, and one each of the genera *Chelonus* and *Microchelonus*), two species of Pteromalidae, and one fly species of Tachinidae.

Study site, experimental layout and fertilization

The experiment consisted of a common garden experiment carried out at a site located 4.3 km east of the locality of Molas, Yucatan, Mexico (20°29′10″N, 89°59′75″W). The site was 10 m a.s.l. and has a warm subhumid climate with summer rains; annual rainfall is 850 mm and the mean annual temperature is 26.2°C (Chico-Ponce de Leon 1999). The organic horizon spans 25 to 50 cm, and soil analyses show that the percent organic matter is 12% (Ortegon-Campos et al. 2012), while nitrogen and phosphorus concentrations are 32 and 18 ppm, respectively (I. Ortegon-Campos unpubl.). The vegetation surrounding the site was composed of patches of secondary tropical dry forest, surrounded by native shrubs and herbs.

During the second week of July 2011, we collected 10 CH fruits from each of 14 *R. nudiflora* (mother) plants belonging to a population located in Subincancab, Yucatan (20°86′33″N, 89°53′24″W; 10.6 km northeast of Molas). We chose this source population because it was large (>1000 plants) and had ecological and soil conditions similar to those of the common garden site at Molas. Because seeds rarely disperse more than 1 m from the parent plant (V. Parra-Tabla upubl.), we sampled mother plants that were at least 2 m apart (with inter-plant distances in many cases being greater than 30 m). Soil conditions were homogeneous within this site, as is the case for *R. nudiflora* populations found at other sites (Ortegón-Campos et al. 2012). All seeds from a given mother plant represented a maternal half-sib family. We germinated seeds the first week of August 2011, and then transplanted them into 1-l plastic bags filled with a mix of native soil and peat moss (1:1). All seedlings were fertilized once with 10 ml of a solution containing N (20%), P (30%) and K (10%), at a concentration of 2 g l^{-1}. In order to minimize maternal effects, bagged plants remained in a nursery under homogenous conditions during a two-month period.

In October 2011, 20 plants from each maternal family were randomly selected and transplanted into a 8×11 m

plot (n = 280; 14 families × 10 replicates × 2 treatments). Distance among rows and among plants within rows was 0.5 m and plant positions were randomized throughout the plot. Subsequently, we established the soil fertility treatment by randomly selecting half of the plants from each family, and watering them twice (last week of October 2011 and first week of November 2011) with 40 ml of the previously mentioned fertilizer solution. This resulted in a deposition of 0.4 g of nitrogen and 0.6 g of phosphorus to an area of ca 400 cm^2 around each plant, and parallels fertilization treatments in past studies that produced effect sizes that were biologically realistic (relative to plant growth responses to natural variation in soil fertility; Gruner et al. 2008). Non-fertilized (control) plants were given 40 ml of water without fertilizer, also twice during the same dates. This amount of fertilization chosen released nutrient limitation sufficiently to cause a significant 1.6-fold increase in leaf production ($F_{1,13} = 19.62$, $p = 0.0006$), which falls within the range of fertilization effect sizes for producer biomass reported by previous studies in terrestrial ecosystems (Gruner et al. 2008). Accordingly, such effect size is also biologically realistic in our study system as it compares to the magnitude of variation observed amongst *R. nudiflora* populations grown with soil from two contrasting sites where this species naturally occurs (1.4 to 1.5-fold difference in above-ground biomass under different soil types; Ortegón-Campos unpubl.). Starting one week after the second fertilizer application, all plants were given 40 ml water three times a week throughout November, twice a week during the first two weeks of December, and once a week for the remainder of December, coinciding with the end of the rainy season.

Measurements of fruit production, seed predator and parasitoid abundance

Plants were monitored throughout November and December 2011, as well as the first week of January 2012. Although this period was after the usual flowering peak for *R. nudiflora*, nearby wild plants flowered throughout the experiment and insect abundances remained high due to rainfall extending into December. For each plant we recorded the number of cleistogamous fruits produced on a weekly basis, and summed counts across all sampling dates for statistical analysis. During each weekly survey, we only counted mature fruits as these stay on the plant less than a week, precluding an overestimation of fruit production by summing across surveys. CH fruit production was very low (< 10% of total fruits produced) and was not analyzed. Therefore, all analyses were based on CL fruit production which we refer to hereafter simply as 'fruit number' or 'fruit production'.

To document seed predator and parasitoid attack, each week we collected up to 10 ripe CL fruits per plant (depending on availability of ripe fruits during each survey) throughout December which is when fruit production peaked, for a total of 10 sampling weeks (mean across all surveys of 7.87 ± 0.31 (SE) fruits collected per plant per survey). We opened each fruit in the laboratory and using a stereoscopic microscope recorded: (a) seed predator attack based on the presence of the larvae or frass, and (b) parasitoid presence and identity based on the presence of a cocoon or the adult parasitoid (Abdala-Roberts et al. 2010). Sampling ripe fruits ensured enough time for both herbivore and parasitoid attack to take place, and was also aimed at allowing more time for both endoparasitic and ectoparasitic parasitoid species to emerge from their hosts and be identified. Based on the specimens found in the sampled fruits, we divided parasitoids into four taxonomic groups ranging from family to genus: Ichneumonidae, Tachinidae, Pteromalidae and *Bracon* sp.

We estimated total abundance of seed predators per plant by multiplying the proportion of attacked fruits (number of fruits with seed predator/total fruits collected) by the total number of fruits counted per plant, while for parasitoids we multiplied the proportion of parasitized seed predators (number of fruits with parasitoids/number of fruits with seed predators) by the estimated total abundance of seed predators per plant. Calculations were based upon insect abundance data pooled across all sampling periods; in the case of parasitoid abundance, we also pooled specimens across all taxonomic groups.

Statistical analyses

Plant family and fertilization effects on fruit, seed predator and parasitoid abundance

We first tested for maternal family, fertilization, and family × fertilization effects on fruit number. Subsequently, to evaluate if these bottom–up effects on fruit number cascade up the food chain, we tested for the same main factors and interaction on seed predator abundance, as well as parasitoid abundance; hereafter we refer to these insect abundance models as 'initial models' (vs 'mechanistic models' below). For the insect abundance models, to inspect what bottom–up force was more important in driving the observed patterns, we compared the strength of maternal family and fertilization effects both in terms of effect sizes expressed as log-response ratios (natural log of the ratio between fertilized and unfertilized plants, or among the two most extreme plant families; Hedges et al. 1999), as well as based on the percent of variation explained by each factor relative to total variation using model sums of squares for untransformed data (i.e. main effect R^2 values; Johnson 2008). For maternal family effects, both measures of effect strength correspond to maternal half-sib effects.

Mechanisms explaining plant family and fertility effects

To assess the mechanisms by which plant genetic and fertilization effects are transmitted to seed predators and parasitoids, we departed from the two initial models for seed predator and parasitoid abundance and constructed two models which included additional terms. For seed predators, we tested for density-mediated effects on herbivores via fruit number by including fruit number as a covariate. In testing for a relationship between seed predator and fruit number, we found a density-dependent pattern (i.e. saturating; see results section). Therefore, the square of fruit number was kept in the model to account for this curvilinear response. If fertilization and family effects on seed predators are due entirely to variation in fruit number (i.e. density-mediated effect), then any significant effects of these factors

observed in the initial models would become non-significant once the effect of fruit number is accounted for. Alternatively, if fertilization or family effects remain significant, this implies an effect independent of those transmitted through fruit number. This model also tested for the fruit number × family and fruit number × fertilization interactions to examine if plant genetic variation and fertilization effects influence the function of plant-seed predator interactions once fruit number was accounted for (i.e. if the rate of change in herbivore number for a given change in fruit number varies among plant families or due to fertilization). Accordingly, we also included the fruit number^2 × fertilization and fruit number^2 × family terms because testing for changes in the function of plant-seed predator interactions across maternal plant families and fertilization levels should account the non-linearity in this resource–consumer relationship. The mechanistic model for parasitoid abundance was the same as that for seed predators, except that instead of fruit number it included seed predator abundance as a covariate to test for density-mediated effects of plant family and fertilization on parasitoids via seed predator abundance. Likewise, this model included the effects of seed predator number × family and seed predator number × fertilization to test for interaction modification effects of plant family and fertilization on parasitoids, respectively. The term seed predator^2 was previously removed from this model owing to its non-significance ($F_{1,177} = 0.14$, $p = 0.70$).

Given that we observed significant variation among maternal families for fruit, seed predator and parasitoid abundance (Results), we performed regressions using family means to formally test whether plant genetic variation in one of these variables was associated with genetic variation in another and, in this case, the function (slope) describing the association. Furthermore, these regressions can also be viewed as tests for density-mediated effects of plant family on seed predators and parasitoids. First, we tested for a relationship between fruit and seed predator number (including fruit number^2 based on the curvilinear relationship observed from the mechanistic model). We also tested for density-dependence in seed predator attack with a regression between the proportion of fruits attacked by the seed predator (fruits with seed predator/total fruits collected) and fruit number. Secondly, we performed a regression between plant family means for seed predator and parasitoid number, and related the proportion of parasitized seed predators to the number of seed predators to test for density dependence in parasitoid attack. Finally, we tested for the indirect association between plant genetic variation for fruit number and parasitoid abundance to assess how parasitoids, in tracking seed predators, indirectly respond to the abundance of the basal resource in the system. For all regression models we only used unfertilized plants, as we sought to test for these genetic associations under unmanipulated soil conditions.

General considerations

All analyses were performed in SAS ver. 9.1. Models for fruit, seed predator and parasitoid abundance (continuous data) were performed with Proc GLM assuming a normal distribution of residuals after log-transforming fruit number data and square-root transforming seed predator and parasitoid data (normality achieved in all three cases). The three models treated plant family and family × fertilization terms as random effects (as well as any other interactions including plant family), and in all cases we report results for type III sums of squares. While generalized linear models and maximum likelihood approaches are frequently favored for non-normally distributed data and random effects (respectively), we did not adopt these approaches here for several reasons. Preliminary analyses with generalized linear models (Proc GLIMMIX) using a negative binomial error (to account for overdispersion) did not converge for some analyses. Likewise, the use of maximum likelihood methods (Proc MIXED) with transformed data was problematic because log-likelihood ratio tests for random effects (i.e. plant family, family interactions with covariates and fertilization) require testing for multiple random effects one at a time. Currently there is little guidance on the appropriate order to perform these tests using MIXED, as well as which fixed terms should remain in the models at each step, and preliminary analyses showed this issue influenced the outcome of our analyses. Consequently, all analyses were performed with general linear models (Proc GLM) and transformed data, specifying the correct F-ratios for random effects with the TEST statement. Except where stated otherwise, we present back-transformed least-square means and standard errors as descriptive statistics. Finally, we used Proc REG to relate family means for fruit number and insect abundances, with residuals being normally distributed in all cases.

Results

Magnitude of plant genetic and soil fertility effects across trophic levels

Fruit number

Significant plant genetic variation was observed for fruit number, with up to three-fold differences among maternal plant families (mean ± SE: 33.50 ± 7.45 to 101.70 ± 10.35 fruits; Table 1). In addition, fertilization caused a significant (but comparatively lower) 1.4-fold increase in fruit number (fertilized = 76.90 ± 3.80; not fertilized = 54.10 ± 3.20 fruits) (Fig. 1). However, the family × fertilization effect was not significant, showing that these two factors acted independently on fruit production (Table 1).

Table 1. Summary of results testing for half-sib family, fertilization and family by fertilization effects on *Ruellia nudiflora* cleistogamous fruit number, seed predator abundance, and parasitoid abundance. df = numerator, denominator degrees of freedom. Significant effects ($p < 0.05$) are in bold.

Response	Source	df	F-value	p-value
Fruit number	Family	13,13	3.84	**0.01**
	Fertilization	1,13.37	20.40	**0.0005**
	Family × Fertilization	13,197	1.09	0.373
Seed predator abundance	Family	13,13	5.43	**0.002**
	Fertilization	1,14.68	86.80	**<0.0001**
	Family × Fertilization	13,197	0.25	0.99
Parasitoid abundance	Family	13,13	4.09	**0.008**
	Fertilization	1,13.68	10.41	**0.006**
	Family × Fertilization	13,193	0.65	0.813

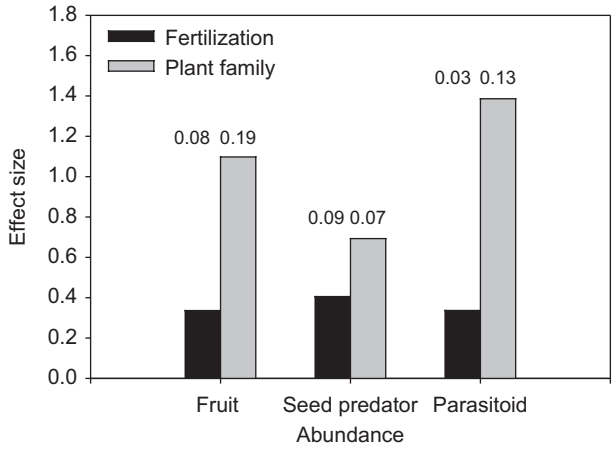

Figure 1. Effect sizes estimated as log-response ratios for *Ruellia nudiflora* maternal family and soil fertilization effects on fruit, seed predator and parasitoid abundance. Values above each bar represent R^2 values for each main effect based on model type III sums of squares.

Seed predator abundance

A total of 2005 fruits were collected, of which 58.3 ± 2.0% (raw mean and SE) were attacked by the seed predator. The initial statistical model showed significant effects of both plant family and fertilization on seed predator number (Table 1). In examining the strength of these effects, we found that maternal families showed up to two-fold differences (range from 22.71 ± 3.48 to 45.42 ± 4.01 seed predators plant^{-1}) and explained 7% of total variation for seed predator abundance in the model ($R^2 = 0.07$) (Fig. 1). In contrast, the effect of fertilization was comparatively smaller, causing a 1.5-fold increase in seed predator abundance (fertilized = 39.94 ± 2.15, unfertilized = 26.41 ± 1.73 seed predators plant^{-1}) (Fig. 1); the percent of variation explained by this effect was similar to that of maternal family ($R^2 = 0.09$) (Table 1; Fig. 1). As with fruit number, family and fertilization had independent effects on seed predators (non-significant family × fertilization interaction; Table 1).

Parasitoid abundance

A total of 655 parasitoid specimens were recorded, of which 84% (550 specimens) belonged to *Bracon* sp., followed by Pteromalidae (4.7%, 31), Tachinidae (1.98%, 13), and Ichneumonidae (0.61%, 4). The remaining specimens (ca 8.7%) were not identified because the parasitoid exited the fruit prior to fruit collection. Across all parasitoid groups, 48.0 ± 2.0% of the recorded seed predator larvae were parasitized. Based on the initial model, we observed significant effects of both plant family and fertilization on parasitoid abundance (Table 1). Maternal families showed up to four-fold differences (8.58 ± 3.35 to 34.10 ± 4.17 parasitoids) and explained 12% of total variation for parasitoid abundance in the model ($R^2 = 0.12$) (Fig. 1). The effect of fertilization was comparatively smaller, causing a 1.4-fold increase in parasitoid abundance (fertilized = 21.16 ± 1.88, unfertilized = 15.36 ± 1.48 parasitoids) and explaining only 3% of the total variation in the model ($R^2 = 0.03$) (Table 1, Fig. 1). As for fruit number and seed predators (see above), plant family and fertilization had independent effects on parasitoids (non-significant family × fertilization interaction; Table 1).

Mechanisms of plant genetic and soil fertility effects on tri-trophic interactions

Plant-seed predator interactions

The mechanistic model for seed predator abundance showed no evidence of plant genetic variation for seed predator abundance after accounting for significant effects of fruit number and fruit number^2 (seed predators = 0.102 × fruit number − 0.0004 × fruit number^2 + 1.54) (Table 2), showing that plant genetic variation for seed predators was mediated by genetic variation for fruit number. Results from this model also showed that the fruit number × family and fruit number^2 × family interactions were not significant, indicating that the rate of change in herbivore number for a given change in fruit number did not vary among maternal families (Table 2). Likewise, in parallel to the ecological trend observed for the mechanistic model, the regression analysis with plant family means showed a positive, saturating

Table 2. Summary of results testing for mechanisms by which *Ruellia nudiflora* maternal family and soil fertilization influence seed predator and parasitoid abundance. df = degrees of freedom. Significant ($p < 0.05$) and marginal ($0.05 < p < 0.10$) results are in bold and italics, respectively. The seed predator^2 term was previously removed from parasitoid abundance model owing to its non-significance.

Response	Source	df	F-value	p-value
Seed predator number	Family	13,178.6	0.53	0.903
	Fertilization	1,177.3	3.02	*0.084*
	Family × Fertilization	13,167	0.61	0.842
	Fruit number	1,167	39.86	**<0.0001**
	Fruit number × Family	13,167	0.82	0.633
	Fruit number × Fertilization	1,167	2.81	*0.095*
	Fruit number^2	1,167	13.24	**0.0004**
	Fruit number^2 × Family	13,167	0.96	0.497
	Fruit number^2 × Fertilization	1,167	3.39	*0.067*
Parasitoid number	Family	13,157.1	1.20	0.282
	Fertilization	1,166.1	5.29	**0.022**
	Family × Fertilization	13,178	0.68	0.782
	Seed predator number	1,178	195.08	**<0.0001**
	Seed predator × Family	13,178	1.16	0.310
	Seed predator × Fertilization	1,178	9.96	**0.001**

(non-linear) relationship, where plant genetic variation for fruit abundance explained 68% of plant genetic variation for seed predator abundance (seed predators = 1.28 × fruit number + 0.0091 × fruit number^2 − 10.58) (Fig. 2a). Because the rate of seed predator abundance declined with increasing fruit abundance, plant family means for the proportion of fruits with seed predator also declined with fruit number, confirming an inverse density-dependent relationship ($R^2 = 0.72$, $p < 0.0001$) (Fig. 2a, inset).

The mechanistic model also showed that the effect of fertilization on seed predator abundance was not significant (albeit marginal) after accounting for fruit number and fruit number^2 (Table 2), suggesting that fertilization effects on seed predator abundance were also mediated by fruit number. Unlike maternal family effects, however, there was a residual (marginal) fertilization effect on seed predators after accounting for fruit abundance (fruit number and fruit number^2; Table 2); this influence interacted with fruit number (marginally significant fruit number × fertilization and fruit number^2 × fertilization terms; Table 2), suggesting that plant-seed predator interactions changed across fertilization treatments. Indeed, a trend towards a greater rate of increase in seed predator number with fruit number was observed for fertilized relative to unfertilized plants (Fig. 3a).

Seed predator–parasitoid interactions

Results from the mechanistic model showed that the effect of plant family on parasitoid abundance became non-significant after accounting for seed predator abundance (Table 2), indicating that plant genetic variation for parasitoid number was mediated by seed predator abundance. The effect of seed predator number on parasitoids was significant (Table 2), showing a positive linear relationship (parasitoid abundance = 0.043 × seed predator number + 3.01). We did not find evidence that plant family modified seed predator–parasitoid interactions (non-significant seed predator number × plant family interaction; Table 2). In parallel to the seed predator–parasitoid ecological association depicted by the mechanistic model, the regression model for plant family means indicated that 68% of plant genetic variation for parasitoid abundance was explained by plant genetic variation for seed predator number (Fig. 2b). The fact that the rate of parasitoid recruitment to seed predators was constant, and not-density dependent, can also be seen in the fact that the proportion of parasitized seed predators was independent of seed predator number ($R^2 = 0.10$, $p = 0.25$) (Fig. 2b, inset). Finally, the combination of the saturating fruit-seed predator relationship and the linear response of parasitoids to seed predators implied a marginally significant saturating relationship between

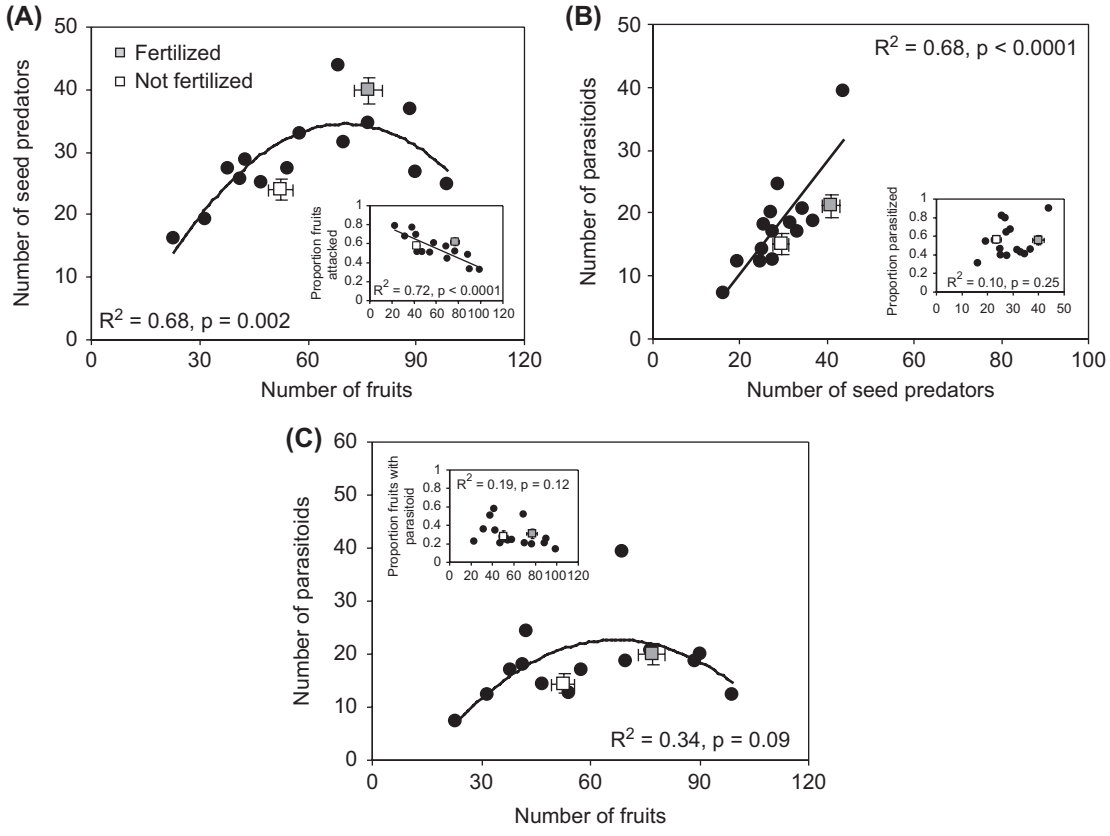

Figure 2. Regressions based on *Ruellia nudiflora* half-sib family means showing (A) the relationship between cleistogamous fruit number and seed predator abundance, (B) between seed predator abundance and parasitoid abundance, and (C) between cleistogamous fruit number and parasitoid abundance. Insets for panel (A) and (B) have as *y*-axes the proportion of fruits with seed predator (fruits with seed predator/total fruit number) and the proportion of parasitized seed predators (parasitized seed predators/seed predator number), respectively. The inset for panel (C) shows the proportion of fruits with parasitoid relative to fruit number. Squares are back-transformed least-square means and SE for each fertilization treatment level.

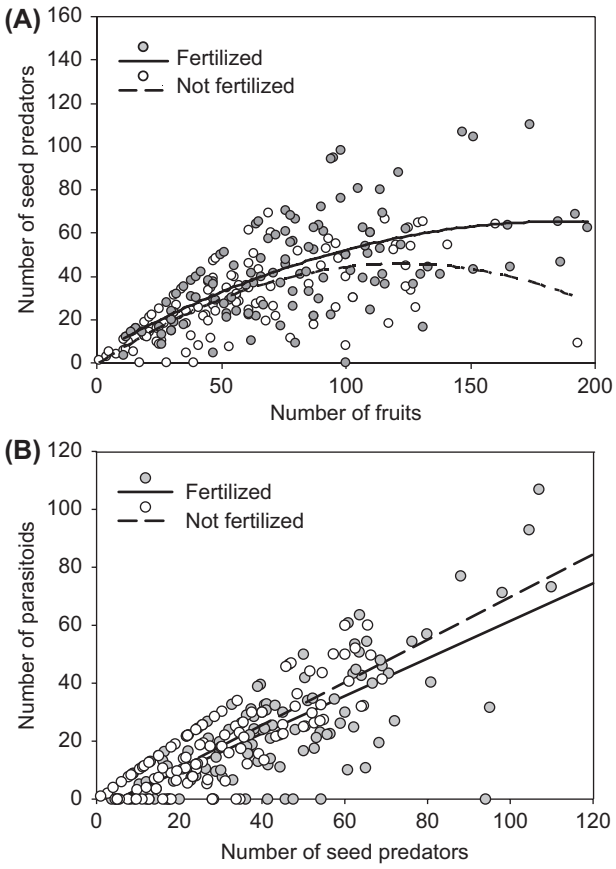

Figure 3. Relationship between (A) number of fruits and number of seed predator larvae, and (B) between number of seed predator larvae and number of parasitoids in fruits of *Ruellia nudiflora*. Regression lines are presented separately for each soil fertilization treatment.

parasitoid and fruit number, where the latter explained 34% of plant genetic variation for parasitoid abundance (parasitoid number $= 1.070 \times$ fruit number $- 0.008 \times$ fruit number$^2 - 13.248$) (Fig. 2c).

Importantly, results from the mechanistic model for parasitoids also revealed a significant interaction between the effects of fertilization and seed predator abundance (Table 2). Specifically, fertilization modified seed predator–parasitoid interactions, such that the rate of increase in the number of parasitoids with the number of seed predators was lower for fertilized (number of parasitoids $= 0.647 \times$ number of seed predators $- 3.29$, $R^2 = 0.54$, $p < 0.001$) relative to unfertilized plants (parasitoids $= 0.740 \times$ seed predators $- 3.90$, $R^2 = 0.72$, $p < 0.001$) (Fig. 3b).

Discussion

Variation in fruit number of *Ruellia nudiflora* fruit number due to both plant genetics and soil fertilization had strong effects on herbivores, which cascaded up to the third trophic level. However, the strength of plant genetic effects on both the seed predator and parasitoids was greater than that of fertilization when measured in terms of effect sizes. This was consistent with estimates of effect strength based on percent of total variation explained, except for seed predator abundance where both factors had a similar explanatory power. Together, these findings support the idea that plant genetic effects on arthropod communities may be as strong or stronger compared to environmental factors (Johnson and Agrawal 2005). Furthermore, the effects of plant genetic variation were based upon half-sib families (vs clones or full-sib families) from a single population, whereas previous data show that the fertilization treatment had a similar magnitude of effect on plant growth relative to that caused by natural variation in soil conditions among sites. Consequently, the observed difference between environment- and plant-genetic effects would have been even greater had we used genotypes from different sites (i.e. population-level genetic variation). Another important result was that plant genetic and fertilization effects operated independently across trophic levels, which contrasts with previous studies showing that plant genotypes interact with biotic (Abdala-Roberts et al. 2012) and abiotic (Stiling and Rossi 1996, Johnson and Agrawal 2005) factors in shaping arthropod communities. This finding suggests a lack of growth-defense or reproduction-defense tradeoffs for the studied *R. nudiflora* genotypes.

In examining the mechanisms mediating these bottom–up effects, we found that variation for resource abundance (i.e. fruit number) due to both maternal family and fertilization affected seed predators and parasitoids via density-mediated effects (i.e. interaction chains), with the shape of these resource-consumer relationships changing across trophic levels. However, these bottom–up factors differed with respect to whether they mediated consumer–resource interactions: whereas soil fertilization mediated higher trophic-level interactions – increasing the number of seed predators per fruit and decreasing the number of parasitoids per seed predator – variation among plant families did not mediate consumer–resource interactions. Together, these findings illustrate that environmental and plant genetic-based variation for resource abundance can independently cascade up food chains to higher trophic levels, and that these sources of variation may differ with respect to the strength and underlying mechanisms of their effects.

Shape of resource–consumer relationships across the food chain

The relationship between fruit and seed predator number approximated a type II functional response (sensu Holling 1959), with a decelerating response of seed predators to fruit abundance (Fig. 1a), such that the proportion of attacked fruits declined with increasing fruit abundance (Fig. 1a, inset). At least three mechanisms may account for the shape of this relationship. First, plant families that produce more fruits may be better defended against seed predators. However, we did not measure plant resistance traits, and thus have no basis by which to test this hypothesis. Second, stronger herbivore suppression by parasitoids on plants with more fruits could have caused an avoidance of ovipositing female moths for plants with large fruit display sizes (i.e. enemy free space; Jeffries and Lawton 1986). Our results do not support this hypothesis as parasitism rates did not vary with herbivore abundance (Fig. 1b, inset).

Finally, plant families producing larger fruit display sizes may have received proportionally less attack due to herbivore satiation. In support of this explanation, larger fruit display sizes in this plant species have been shown to maximize fruiting synchrony (because plants with larger display sizes tend to bear fruits for longer periods of time, which maximizes temporal overlap in fruiting with neighboring plants) and thus lead to herbivore satiation (V. Parra-Tabla unpubl.; reviewed by Elzinga et al. 2007).

In contrast to seed predators, parasitoid number increased linearly with seed predator abundance (Fig. 1b), yielding a type I density-independent functional response (Holling 1959) where the proportion of parasitized seed predators was unrelated to seed predator abundance over a wide range of herbivore densities (Fig. 1b, inset; reviewed by Stiling 1987, Walde and Murdoch 1988). The observed difference between parasitoid and seed predator functional responses may be due to contrasting foraging behavior and efficiency (Schädler et al. 2010). Indeed, while satiation may have influenced seed predator attack, factors such as egg-laying or handling time (typically yielding parasitoid negative density-dependence) apparently did not influence parasitism rates during this study and thus did not lead to parasitoid satiation. Overall, these results suggest that a mechanistic understanding of bottom–up effects on multi-trophic interactions requires an explicit examination of the shape of resource–consumer relationships across trophic levels (e.g. tests for non-linearity).

Plant genetic effects on tri-trophic interactions

Plant genetic variation for fruit number cascaded up the food chain via density-mediated effects by influencing the abundance of seed predators (directly) and parasitoids (indirectly). This finding agrees with previous work showing density-mediated indirect effects of plant genotype on herbivores and predators (Bailey et al. 2006, Johnson 2008, Mooney and Agrawal 2008). In addition, some of these studies have also shown that plant genotypes mediate interactions at higher trophic levels, presumably through changes in traits among the interacting species (Mooney and Agrawal 2008). However, our findings suggest that *R. nudiflora* genetic effects did not operate in this manner as the function of plant-seed predator or seed predator–parasitoid interactions remained unchanged across maternal families. It is important to note, however, that even though plant-seed predator interactions did not differ among families (i.e. non-significant family × fruit number effect on seed predators), the non-linear relationship between fruit and seed predator number intrinsically leads to differences among plant genotypes in the rates of attack by seed predators: families producing few fruits experience stronger per capita effects of seed predators (i.e. more herbivores per fruit), while those producing more fruits receive lower per capita effects (Fig. 1a). This finding suggests that seed predators will select for plant genotypes with larger display sizes.

We observed stronger overall effects of plant genetic variation on parasitoid abundance (four-fold, $R^2 = 0.12$) compared to seed predator abundance (two-fold, $R^2 = 0.07$). This contrasts with previous studies showing that plant genotype and species effects are stronger on herbivores than on predators, presumably because the former respond more strongly to genetically-based trait variation in plants (Johnson and Agrawal 2005, Scherber et al. 2010). Hence, our results suggest that predators and parasitoids may be more responsive to plant genetic variation than previously thought (e.g. when plant traits directly influence predator foraging; Hare 2002).

Soil fertilization effects on tri-trophic interactions

As for plant family effects, fertilization also operated via density-mediated effects across trophic levels by increasing herbivore abundance and, in turn, parasitoid abundance (Stiling and Rossi 1996, Bridgeland et al. 2010). In contrast to plant genetic effects, however, fertilization effects acted through interaction modification by increasing the rate of seed predator attack on fruits (marginally significant) and decreasing the rate of parasitoid attack on seed predators. Indeed, the fact that fertilization weakened parasitoid effects on seed predators may have led to greater seed predator attack rates on fertilized plants, presumably through a behavioral response in which seed predators sought to escape stronger enemy effects on unfertilized plants. It is likely that lower parasitism rates on fertilized plants resulted from a change in plant or herbivore traits indirectly influencing parasitoids (i.e. trait-mediated indirect effect; Strauss and Irwin 2004, Ohgushi 2005). Specifically, we speculate that faster development of herbivores in fruits from fertilized plants lowered the probability of parasitoid attack, whereas slower larval development facilitated detection by parasitoids and resulted in higher parasitism rates on unfertilized plants (i.e. slow-growth, high-mortality hypothesis; Williams 1999, Mooney et al. 2012). This argument is supported by the fact that parasitoid species in this system attack eggs or first instar larvae of the seed predator, and that seed predator larvae complete their development in less than a week. Thus, slight changes in herbivore larval developmental time may have strong effects on the opportunity for parasitoid oviposition during these early stages.

Conclusions

Our findings show that plant phenotypic effects cascading up the food chain may differ depending on whether the underlying source of variation are plant genetics or the environment. We found that maternal plant family effects were transmitted to the third trophic level, were consistent across differing soil fertility environments, and were generally stronger than fertilization effects. These results emphasize the importance of plant genotype as a structuring force of arthropod communities relative to environmental factors. Our findings also show that plant genotype and environmental effects may independently cascade up food chains as well as influence species interactions through contrasting mechanisms. Based on these results, we conclude that understanding the strength and nature of bottom–up effects is as important as determining the overall magnitude of plant trait variation influencing multi-trophic communities. Furthermore, in comparing environmental and plant genetic

effects on higher trophic levels, this work builds towards a more complete understanding of the tri-trophic consequences of plant genetic variation.

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