

## NATURAL SELECTION ON EXTRAFLORAL NECTAR PRODUCTION IN *CHAMAECRISTA FASCICULATA*: THE COSTS AND BENEFITS OF A MUTUALISM TRAIT

MATTHEW T. RUTTER<sup>1</sup> AND MARK D. RAUSHER

Department of Biology, Duke University, Durham, North Carolina 27707

**Abstract.**—Cost-benefit models of the evolution of mutualism predict that the current state of mutualism results from trade-offs between fitness costs of mutualist traits and the fitness benefits of association. We test the assumptions of such models by measuring patterns of natural selection on a mutualist trait, extrafloral nectar production in *Chamaecrista fasciculata*. Selection was measured on plants from which ants had been excluded (removing the mutualist benefit of the trait), from which all insects had been excluded (removing costs of herbivory in addition to mutualist benefits), and unmanipulated plants (where both costs and benefits were present). Selection analysis based on half-sibling-mean regressions of fitness on the trait revealed no evidence of costs of extrafloral nectar production in the absence of all insects or in the absence of ants. However, examination of the selective surfaces for these treatments suggest that costs of nectar production may exist and are exacerbated by the presence of herbivory. In the presence of ants, natural selection favors high extrafloral nectar production, consistent with a fitness benefit to this mutualist trait in the presence of the mutualist partner. In this study, the interaction of costs and benefits did not produce an evolutionary optimum for the trait within the range of variation observed, suggesting that application of a cost-benefit framework to this trait will benefit from considering the influence of temporal and spatial variation on the quality of costs and benefits.

**Key words.**—Ant-plant interactions, cost-benefit models, extrafloral nectar, mutualism, selection analysis.

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Mutualisms are interactions between species in which both participants have higher fitness when they interact than when they do not. Mutualisms are classic examples of coevolution (Janzen 1966; Gilbert and Raven 1975; Futuyma 1998) and have been described as “the most omnipresent of any organism-to-organism interaction” (Janzen 1985). Despite the ubiquity of these interactions, theoretical and mechanistic understanding of mutualisms has progressed relatively slowly (Bronstein 1994a), because ecological and evolutionary studies of species interactions have focused primarily on antagonistic interactions such as predation and competition.

A major approach to understanding the evolution of interactions involves evolutionary cost-benefit models, in which the evolution of a trait (or interaction) is described in a framework of fitness “costs” and “benefits.” Although the cost-benefit approach provides a straightforward conceptual framework for understanding the evolution of adaptive traits involved in mutualisms (Bronstein 1994a, 1998), a number of uncertainties associated with this approach have seldom been addressed. One major concern is that it is often unclear whether there are costs associated with adaptations involved in mutualisms. Costs are often difficult to demonstrate because they may be highly context dependent (Bronstein 1994b), and it is often hard to separate the “costs” of a trait from its benefits.

A second concern is that even if traits associated with mutualisms have costs, it is unclear how frequently costs and benefits balance to produce an intermediate optimum recognizable by the operation of stabilizing selection. Although rarely stated explicitly, a logical conclusion often derived from models of mutualism is that trait values are subject to stabilizing selection, because these models either explicitly

or implicitly predict that populations will reach stable equilibrium trait values (Roughgarden 1975; Keeler 1985, 1989; Pierce et al. 1987; Connor 1995; Doebeli and Knowlton 1998; Schwartz and Hoeksema 1998; Ferdy et al., 2002; West et al. 2002; Bergstrom and Lachman 2003). Few empirical studies have examined this prediction directly, and although the benefits of mutualism traits have frequently been demonstrated (reviewed in Bronstein 1994a), they are rarely, except in the pollination literature (e.g. Holland and Fleming 1999), compared to explicitly measured costs.

Even if selective optima exist, it is not clear how close populations are to these optima. For example, if the optimal trait value varies over time because of fluctuation in costs and benefits, populations may seldom reach the current optimum and will be consistently subject to directional, in addition to stabilizing, selection (e.g., Tiffin and Rausher 1999). Studies of selection on floral traits (the only mutualistic character in which the pattern of selection has been measured) rarely detect stabilizing selection (Herrera 1993), whereas they frequently detect directional selection (Schemske and Horvitz 1989; Campbell 1996; Conner et al. 1996; Caruso 2001). This suggests that these mutualism traits are seldom at their selective optima or that the stabilizing selection is sufficiently weak that only extremely large experiments have sufficient statistical power to detect it. However, it is unclear whether this conclusion holds for other types of traits in other types of mutualisms.

In this study, we address these issues by attempting to determine whether a cost-benefit framework is appropriate for understanding the evolution of an important quantitative trait mediating ant-plant mutualisms: the volume of extrafloral nectar produced by a plant. Many species of plants secrete nectar outside of flowers that attracts ants and other predators (reviewed in Elias 1983). Previous investigations of the adaptive significance of extrafloral nectaries have fre-

<sup>1</sup> Present address: Department of Biology, University of Maryland, College Park, Maryland 20742; E-mail: rutter@wam.umd.edu.

quently demonstrated that ants attracted by extrafloral nectar decrease the number of herbivores present and thereby increase plant fitness (Bentley 1977), although extrafloral nectar is not always a guarantee of effective ant guardianship (O'Dowd and Catchpole 1983; Tempel 1983; Freitas et al. 2000). However, this type of evidence leaves unanswered not only many of the questions raised above, but also a fundamental question about the biological significance of extrafloral nectar production. In addition to attracting ants, a number of other possible physiological and ecological functions have been suggested for extrafloral nectar, including releasing excess sugars (Frey-Wyssling 1955; Helder 1958; Mound 1962; Milburn 1975) and attracting insect parasitoids (Pemberton and Lee 1996; Stapel et al. 1997, 2000). Thus, it is not clear whether attracting ants is the primary benefit associated with nectar production. The only way of answering this question is by experimentally manipulating the presence of ants and determining the effect on the pattern of selection on extrafloral nectar production. If in the absence of ants there are other benefits to nectar production, they can be revealed by the presence of stabilizing selection or directional selection favoring increased nectar production. By contrast, if removal of ants leads to directional selection favoring elimination of nectar production, it can be concluded that attraction of ants is crucial for the evolutionary maintenance of extrafloral nectar.

## MATERIALS AND METHODS

### *Study system*

*Chamaecrista fasciculata* (Fabaceae), the partridge pea, is a native annual found throughout the eastern United States and south into Central America. *Chamaecrista fasciculata* grows in disturbed, sandy areas such as roadsides. In North Carolina, *C. fasciculata* germinates in March–May, flowers in July–September, and produces fruit from September to October. Beginning with the third or fourth true leaf, a saucer-shaped extrafloral nectary can be found at the base of each petiole. These nectaries are 0.5–4 mm across, and secrete up to three microliters of nectar a day. Because almost every leaf has one nectary, there can be more than 200 nectaries on a large plant. Partridge peas are visited by many different ant species throughout their range (Barton 1986; Kelly 1986; pers. obs.). *Chamaecrista fasciculata* is exclusively buzz pollinated by bees, is predominantly outcrossing, (Lee and Bazzaz 1982; Fenster 1991) and the flowers have no nectar. Fruits mature 3–3.5 months after germination.

### *Experimental design*

A base population was established from plants collected from a field in Durham County, North Carolina, in the fall of 1997 and was maintained under greenhouse conditions for four generations. To generate experimental seeds, we employed a paternal half-sibling breeding design in which 33 plants from the base population served as pollen parents (sires). Each of these plants was crossed to three randomly selected seed parents (dams). Flowers of seed parents were emasculated to prevent self-pollination and were hand pollinated. Because of a limited number of plants, some dams

were mated to more than one sire. As a result, the 99 maternal full-sib families are represented by only 64 maternal genotypes. This resulting design is thus an incomplete paternal half-sib design, which was analyzed as a half-sib design (Lynch and Walsh 1998; Tiffin and Rausher 1999).

On May 5, 2000, 12 scarified seeds from each maternal full-sib family (1188 total seeds) were planted in flats in the greenhouse. When these plants had developed their first true leaf, they were transplanted, in two spatial blocks, into a previously plowed and disked field in the Duke Forest in Durham County, North Carolina. Within each block, seeds were planted 50 cm apart within rows, with 100 cm between rows. Weeds were removed from the field weekly during the first six weeks after planting to minimize competition and assure that experimental plants became established. The field was surrounded by an electric fence and chicken wire to exclude mammals such as deer and rabbits. Transplanting began on May 20 and was completed by June 25. Spatial blocks were planted consecutively; transplant date did not have a significant effect on final plant size and all plants senesced before the first frost. Plants began flowering by July 15 and seeds began maturing August 10.

Within each block, two plants from each maternal family were assigned to each of three treatments: control plants, plants from which ants were excluded, and plants from which all insects, including herbivores, were excluded. Plant location was randomized within each block with respect to maternal and paternal family as well as treatment. For all plants, a circle of approximately 25 cm was weeded around the plant to prevent contact between the experimental plant and the native vegetation. For control plants, there was no other manipulation. For ant-exclusion plants, all ants were manually removed from the plant. A  $9 \times 9$  cm<sup>2</sup>-foil square was then placed around the base of the plant coated with a layer of Tanglefoot (Tanglefoot Co., Grand Rapids, MI), a highly sticky substance that is extremely difficult for ants to cross. However, most insects, including the generalist orthopterans, weevils, and lepidopterans, which were the most common herbivores in this field, were still able to access these plants, presumably by flight or jumping onto the plant. The Tanglefoot squares were frequently replaced as they became clogged with debris, and plants were periodically monitored to ensure that ants did not access the plants. The effectiveness of this treatment was verified by snapshot surveys: plants with Tanglefoot had significantly fewer ants than plants without Tanglefoot (mean of 0.89 and 2.94 ants/plant with and without Tanglefoot, respectively;  $n = 85$  observations per treatment,  $P < 0.003$ ). Plants that were in the insect exclusion treatment were treated with the Tanglefoot treatment as above, and were additionally sprayed with the insecticide Sevin (Bayer Crop Science, Research Triangle Park, NC) once every two weeks.

### *Trait measurement*

The volume of extrafloral nectar produced per nectary over a 24-h period was measured during a period from July 15 to August 15. For control plants, ants were removed from the plant, and a Tanglefoot square was temporarily placed under the plant for the measurement period. All standing extrafloral

nectar was removed with a piece of filter paper from the 10 nectaries in the highest vertical position on the plant. Twenty-four hours later, all newly accumulated extrafloral nectar from those 10 nectaries was absorbed onto a single filter paper wick made of Whatman #1 filter paper (Whatman, Inc., Clifton, NJ). The area of the spot made by the nectar was estimated from a digital image using the computer program Image Pro Plus (ver. 4.1 for Windows, Media Cybernetics, Silver Spring, MD). The volume of nectar collected is proportional to the area of the nectar spot (Kearns and Inouye 1993). Nectar production is well documented to be an environmentally variable trait (Wyatt et al. 1992; Campbell 1996; Leiss et al. 2004), and it is possible that individual measures of extrafloral nectar production were influenced by environmental factors. However, the half-sib analyses described below depend primarily on mean nectar production of many individuals that share a particular sire and thus are robust to any particular local environmental factor.

Fruits were collected as they matured until November 1, by which time all plants had senesced. Genetic correlations between flower number, fruit number, and total seed production were positive, strong, and highly significant. For all analyses, the number of viable seeds produced by each plant was used as a measure of fitness, and using flower or fruit number as a fitness measure produced qualitatively identical results. We considered seed viable if they were full in form with a hardened seed coat and did not count shriveled or flattened seed because these rarely germinate. Plant size was measured on all plants at death or senescence by counting the total number of leaves produced (the leaf scars could be counted). Additionally, all plants were censused once in October for visible signs of herbivory, e.g., physical evidence of chewing damage to leaf, stem, flower, or fruit tissue. A dichotomous variable was scored for each plant, registering either the presence or absence of herbivory. This measure is admittedly crude and only represents an estimate of herbivore damage at a single point in time.

Of the 1188 plants planted, 831 survived until the first nectar measurements. When included in the analyses below, plants that did not survive until these measurements were assigned a fitness of zero and the mean nectar volume trait value of their family unit, allowing an estimate of any cost of nectar production that decreased early plant survival.

#### *Data analysis: genetic variation for and correlations among traits*

Tests for the presence of genetic variation for all plant characters other than fitness were carried out on data from those plants that survived until nectar measurement ( $n = 831$ ). Furthermore, because the pattern of plant death led to some change in the family structure of the plants in the field, the dataset was further winnowed to remove dams that were not represented by at least two plants in each treatment and sire combination, and to remove sires represented by only a single dam in any treatment. After reducing the dataset for the test of genetic variation, 661 individuals remained. The test for genetic variation for fitness used all 1188 plants, including those that did not survive to reproduce.

To test for additive genetic variation for most measured

characters, the MIXED procedure of the SAS statistical software package (ver. 8, SAS Institute 2000) was used to perform a mixed-model restricted maximum-likelihood (REML) analysis. The volume of extrafloral nectar per nectary (hereafter "nectar volume") was nonnormal (it was highly right skewed) and resisted most transformation; consequently, a threshold transformation was used for this analysis only (as described in Roff 2001). Because this transformation results in a dataset with a binomial error distribution, the GLIMMIX macro in SAS (2000) was used, using a binomial distribution and a probit link function. Fitness data (total seed set, which equaled zero for plants that died before setting seed) was log transformed to enhance normality, whereas data on plant size and shape were untransformed. Because we are not estimating specific genetic parameters, and only wish to assess whether there is genetic variation for these characters, these transformations are appropriate (Mitchell-Olds and Shaw 1987). Block, pollen parent (sire), seed parent (dam, nested within sire), and the interactions between sire and treatment and between dam and treatment were considered random effects, whereas treatment was considered fixed. In all tests, the significance level of a random effect was determined by the likelihood-ratio statistic, computed by calculating the difference between the  $-2$  REML log-likelihood for the model containing the random effect and the  $-2$  REML log-likelihood for the model without the random effect. The  $P$ -value for this statistic can be determined by a one-tailed, chi-squared test with one degree of freedom (Self and Liang 1987; Littell et al. 1996). A significant sire effect for a character is taken to indicate the presence of genetic variation for that character.

The GLM procedure of SAS (2000), applied to ranks of family means, was used to determine if treatment had a significant effect on the rank of families in nectar volume produced per nectary. The CORR procedure of SAS (2000) was used to calculate pairwise Pearson family-mean correlation coefficients and their significance levels between all measured variables. For this analysis, residuals were used after removal of block effects.

#### *Data analysis: herbivory*

To determine the relationship of the treatments, family lines, and plant size on herbivory, both the MIXED and LOGISTIC procedures of SAS (2000) were performed to detect effects of sire, dam, treatment, and leaf number. Because herbivory was recorded as a dichotomous variable, logistic regression is more appropriate than GLM (Allison 1999). The MIXED procedure of SAS was used with the GLIMMIX procedure to test for effects of sire, dam, treatment and leaf number on herbivory. Because random effects of sire and dam were not significant, the LOGISTIC procedure was then used to examine the fixed effects of treatment and leaf number on herbivory.

#### *Selection analysis*

The pattern and magnitude of selection on nectar volume produced per nectary and leaf number was estimated using genetic selection analysis on family means (Rausher 1992; Stinchcombe et al. 2002). Family means of relative fitness,

TABLE 1. Results of mixed-model restricted maximum-likelihood analysis demonstrating genetic variation for the volume of extrafloral nectar produced per nectary, the total leaf number, and fitness (total lifetime seed set). The analysis of variation in fitness was conducted for the entire dataset. The analyses of variation in nectar volume per nectary and leaf number were conducted on a smaller dataset as described in the text. \*Inclusion of these factors had no effect on the model and are not significant. CPE: covariance parameter estimate.

Fixed Effects						
Trait	Factor	Numerator degrees of freedom	Denominator degrees of freedom	<i>F</i>	<i>P</i>	
Volume of extrafloral nectar per nectary	Treatment	2	48	0.03	0.9721	
Total leaf number	Treatment	2	48	1.45	0.2457	
Fitness	Treatment	2	64	0.65	0.5265	
Random Effects						
Random effect	Volume of nectar/nectary		Total leaf number		Fitness	
	CPE	<i>P</i>	CPE	<i>P</i>	CPE	<i>P</i>
Block	0.75	<0.0001	221.52	<0.0001	0.026	<0.0001
Sire	0.38	0.0098	77.90	0.0021	0.0029	0.017
Dam(sire)	0.58	<0.0001	5.15	0.4043	0.0044	0.0004
Treatment × Sire	0*	ns	10.77	0.2852	0*	ns
Treatment × Dam(Sire)	0*	ns	0*	0*	0*	ns

the dependent variable, were calculated by first dividing the number of viable seeds produced by each plant by the mean viable seeds production of all plants and all treatments, then using the resulting dataset to compute mean seed production within each family and treatment. The effects of block were removed and the residuals were standardized to a mean of zero and averaged over individuals in each family. To evaluate whether the presence of ants and insect herbivores (treatment) altered the overall pattern of selection, we utilized standard analysis of covariance to evaluate the significance of interaction terms involving treatment and the measured characters (Mauricio and Rausher 1997; Juenger and Bergelson 1998; Stinchcombe and Rausher 2001). In both the selection analyses and the analysis of covariance, significance of linear terms was determined from a model containing only linear terms, whereas the significance of quadratic terms was computed from the full model containing both linear and quadratic terms (Lande and Arnold 1983).

#### Visualizing the pattern of selection

The statistical analysis of selection as described above tests for general differences between treatments in the overall pattern of selection. However, because fitness surfaces may often be more complicated than can be described by a second order regression, such analyses alone may not reveal many details of how those patterns differ. To compare in more detail the patterns of selection in the different treatments, we used a nonparametric spline procedure to visualize the selective surfaces associated with each treatment (Schluter and Nychka 1994; Mauricio and Rausher 1997). In particular, we used the G3GRID procedure of SAS (2000) to generate selective surfaces. This procedure interpolates a function (e.g., fitness) of two variables (e.g., nectar volume and leaf number) onto a rectangular grid using a smoothed estimate of a bivariate spline (SAS Institute, vers. 8.02, 2000). As in the previous analysis, family means of residuals of fitness and trait values after the effects of block had been removed were used to create the fitness surface. The ranges of nectar volumes and

leaf number were determined by the maximum paternal half-sib family mean value for this population.

This procedure was used to construct a fitness surface for each treatment. Because we are particularly interested in the location of the peak within each of the surfaces, we performed a bootstrap analysis to create a 95% confidence interval around the maximum point on the surface (Schluter and Nychka 1994). We ran 10,000 replicates of the bootstrap for each treatment, using the maximum value of the surface generated by G3GRID as the test statistic.

Comparing the fitness surfaces across the different treatments reveals how fitness surfaces change when components (e.g., herbivores and ants) are added to the community. Moreover, the actual pattern of selection imposed by each of those components is simply the change in the fitness surface as that component is added, and was calculated as the difference in fitness between two fitness surfaces at each grid point (Mauricio and Rausher 1997). To create confidence intervals for both the maximum and minimum differences between surfaces, we employed bootstrap analyses that jointly estimated the two surfaces being compared, computed the difference between those surfaces, and found the maximum and minimum differences between the surfaces. For each of the two comparisons presented, we ran 10,000 replicates of the bootstrap, using the maximum and minimum differences between the surfaces as the test statistics, and calculated 95% confidence intervals for these statistics.

## RESULTS

#### Genetic variation and correlation between traits

To apply genetic selection analysis, measured characters must be genetically variable (Rausher 1992). Consequently, we first demonstrate that fitness, nectar volume per nectary, and leaf number were genetically variable in our experimental population. Genetic variation is indicated by the significant sire and dam effects for nectar volume ( $h^2 = 0.33$ ) and fitness ( $h^2 = 0.19$ ), and the significant sire effect on leaf number ( $h^2 = 0.14$ ) (Table 1). Block effects were significant for all

TABLE 2. Pearson's correlation coefficients for measured traits. Significance values are directly beneath the correlation coefficient. All correlations are of family means.

Trait	Nectar volume	Relative fitness
Leaf number	0.22892	0.59616
	0.0227	<0.0001
Nectar volume		0.35824
		0.0003

three characters, indicating an effect of local environment. Treatment by sire and treatment by dam effects were not significant for any of the traits, indicating that the ordering of families was the same in all treatments. To our knowledge, this is the first documentation of a heritable basis for extrafloral nectar production (Mitchell 2004).

For all three pairs of characters, family mean correlations were moderate in magnitude and statistically significant (Table 2). Because of these correlations, a multivariate selection analysis must be used rather than separate analyses of selection on nectar volume and leaf number.

*Treatment effects on herbivory and fitness*

Treatments differed significantly in the frequency of herbivory (Table 3A, treatment effect). As expected if ants in our experiments protected plants from herbivores, plants in the ant-exclusion treatment experienced a greater amount of herbivory than either control plants or plants from which ants and insect herbivores were excluded (Table 3A, B). Plants with more leaves also experienced significantly more herbivory (Table 3B, leaf number effect). When this is accounted for, plants receiving herbivory had lower fitness than plants without herbivory, but this did not reach statistical significance ( $P < 0.08$ , Table 3C). These results confirm that the experimental manipulations did affect herbivory levels in the

expected direction, although it cannot be confirmed that herbivory lowered fitness. Surprisingly, the plants in the ant-exclusion treatment were actually largest and had the highest fitness, although this difference between treatments was not significant when family information was included in the model (Table 1).

*Selection analysis*

In all three treatments, directional selection favoring increased leaf number was significant (Table 4). By contrast, significant directional selection on nectar volume was detected only in the control treatment (ants and herbivores present), where selection favored an increase in nectar volume per nectary. Stabilizing/disruptive selection was not detected on either character in any treatment, but significant positive correlational selection occurred in the control treatment. Analysis by treatment thus suggests that the pattern of selection on nectar volume, as indicated by the directional selection gradients on this character and by the correlational selection gradient, differs between at least the control treatment and the two other treatments.

This difference was confirmed statistically using analysis of covariance. In particular, the significant nectar volume  $\times$  treatment effect demonstrates that the magnitude of directional selection on nectar volume differs between treatments (Table 5). Additionally, the nectar volume  $\times$  leaf number  $\times$  treatment effect is significant, indicating that treatments differ in the magnitude of correlational selection. Specific contrasts for both of these selection gradients detected significant differences between the ants-removed treatment and the control (significant for the nectar by leaf number by treatment interaction and marginally significant ( $P < 0.08$ ) for the nectar by treatment interaction), but did not detect significant differences between the removal treatments or between the ants-and-herbivores-removed treatment and the control. The

TABLE 3. The effects of treatment on herbivory, and the effects of herbivory on fitness. (A) Treatment means of herbivory measures, total seed number, and leaf number for censused plants. Standard errors are in parentheses. Herbivory means are equivalent to the frequency with which herbivory was observed in that treatment and are not presented with standard errors. (B) Test for the effect of treatment and leaf number on the occurrence of herbivory. (C) Test of the effect of herbivory and total leaf number on fitness.

A.				
Character	Treatment	Mean	(SE)	
Herbivory	Control	38.80		
	Ants excluded	44.52		
	All insects excluded	36.47		
Seed produced	Control	59.26	(6.13)	
	Ants excluded	78.54	(8.14)	
	All insects excluded	59.90	(6.43)	
Leaf number	Control	25.66	(1.76)	
	Ants excluded	30.06	(2.12)	
	All insects excluded	24.44	(1.68)	
B. Type III analysis of effects				
Effect	df	Wald $\chi^2$	Pr > $\chi^2$	
Treatment	2	6.2890	0.0431	
Leaf number	1	136.2032	<.0001	
Leaf number $\times$ treatment	2	5.4693	0.0649	
C.				
Source	df	Type III SS	F	Pr > F
Herbivory	1	30.28040	3.11	0.0781
Leaf number	1	17668.26564	1816.17	<.0001

TABLE 4. Standardized selection gradients for nectar volume and leaf number. Directional ( $\beta$ ) and stabilizing/disruptive ( $\gamma$ ) selection gradients from selection analysis. Standard errors are in parentheses.

Treatment	Ants and herbivores present (controls)		Ants excluded		All insects excluded	
	$\beta$	$\gamma$	$\beta$	$\gamma$	$\beta$	$\gamma$
Nectar volume	0.695* (0.244)	-0.053 (0.382)	-0.096 (0.165)	-0.042 (0.237)	0.127 (0.119)	-0.292 (0.209)
Leaf number	0.895** (0.209)	0.151 (0.289)	1.294*** (0.224)	0.878† (0.472)	0.646* (0.009)	-0.070 (0.370)
Correlation selection ( $\delta$ )		1.991* (0.703)		-0.618 (0.382)		0.475 (0.600)

†  $P < 0.1$  \*  $P < 0.01$ ; \*\*  $P < 0.001$  \*\*\*  $P < 0.0001$ .

magnitude of directional selection on leaf number may also have differed among treatments, though the leaf number  $\times$  treatment effect was significant only at the  $P < 0.1$  level. Overall, these differences suggest that ants and natural enemies alter the pattern of selection on nectar volume and leaf number.

#### Visualizing the pattern of selection

The partial regression method used above can reveal gross distinctions between the effects of natural selection in different treatments, but does not necessarily provide specific details about how the treatments alter the selective surface because the complexity of the shape of the selective surface may not be captured by a simple second order fitted surface. We have therefore used a bivariate spline approach (Schluter and Nychka 1994) to create a better description of the selective surfaces (Mauricio and Rausher 1997). We consider each treatment in turn in the following paragraphs.

#### Selection in absence of ants and herbivores

A cost of nectar production should be reflected in selection against nectar production in the absence of ants and herbivores (insecticide treatment). In addition, the previous selection analyses suggested that in this treatment, selection favors plants with more leaves. Together, these two patterns of selection would produce a peak in the fitness surface in the upper left corner of Figure 1A, corresponding to minimal nectar volume and maximal plant size.

The 95% confidence region for the actual peak of this

fitness surface is consistent with this expectation. The fitness surface slopes upward toward high leaf number, and the confidence region includes only the largest plants. However, although this confidence region includes the point corresponding to maximal plant size and minimal nectar production, it is very broad with respect to nectar volume, and does not rule out the possibility that the fitness peak corresponds to an intermediate value of nectar production. In the region of the fitness maximum, the fitness contours are essentially flat with respect to nectar production, indicating that any physiological costs of nectar production that exist are minimal. This result is consistent with the failure to detect significant directional selection on nectar volume in this treatment.

#### Selection in the presence of herbivores

Addition of herbivores (ant-exclusion treatment) alters some aspects of the selective surface for extrafloral nectar volume and leaf number (Fig. 1B). The fitness surface in this treatment has an apparent peak corresponding to maximal plant size but minimal nectar production. The 95% confidence region for this peak is smaller than in the insecticide treatment, and is fully consistent with selection favoring low nectar volumes (the confidence interval extends to 2.74 on the nectar volume axis in Fig. 1A and to 1.83 on the nectar volume axis in Fig. 1B). Although the finding of a fitness peak for zero nectar volume may seem inconsistent with our failure to detect significant directional selection on nectar volume in this treatment (see above), it should be noted that in the region of the selective surface in which the population

TABLE 5. Analysis of covariance for relative fitness of *Chamaecrista fasciculata* grown in the field in three treatments. The treatments included plants sprayed with insecticide, plants from which ants were excluded, and control plants. This table combines two analyses: results for linear terms derive from an analysis using linear terms only, whereas results for quadratic terms are derived from an analysis including both linear and quadratic terms (following Lande and Arnold 1983).

Source	df	Type III SS	F	Pr > F
Nectar volume	1	1.72301474	5.23	0.0245
Leaf number	1	19.56990815	59.68	<.0001
Treatment	2	0.15072747	0.23	0.7960
(Nectar) <sup>2</sup>	1	0.17558695	0.59	0.4459
(Leaf number) <sup>2</sup>	1	0.63105976	2.11	0.1503
Nectar $\times$ leaf number	1	0.93868050	3.14	0.0803
Leaf number $\times$ treat.	2	1.56586938	2.38	0.0987
Nectar $\times$ treat.	2	2.48483221	3.77	0.0267
(Nectar) <sup>2</sup> $\times$ treat.	2	0.20776339	0.35	0.7078
(Leaf number) <sup>2</sup> $\times$ treat.	2	0.87486679	1.46	0.2379
Nectar $\times$ leaf number $\times$ treat.	2	3.36560087	5.62	0.0052

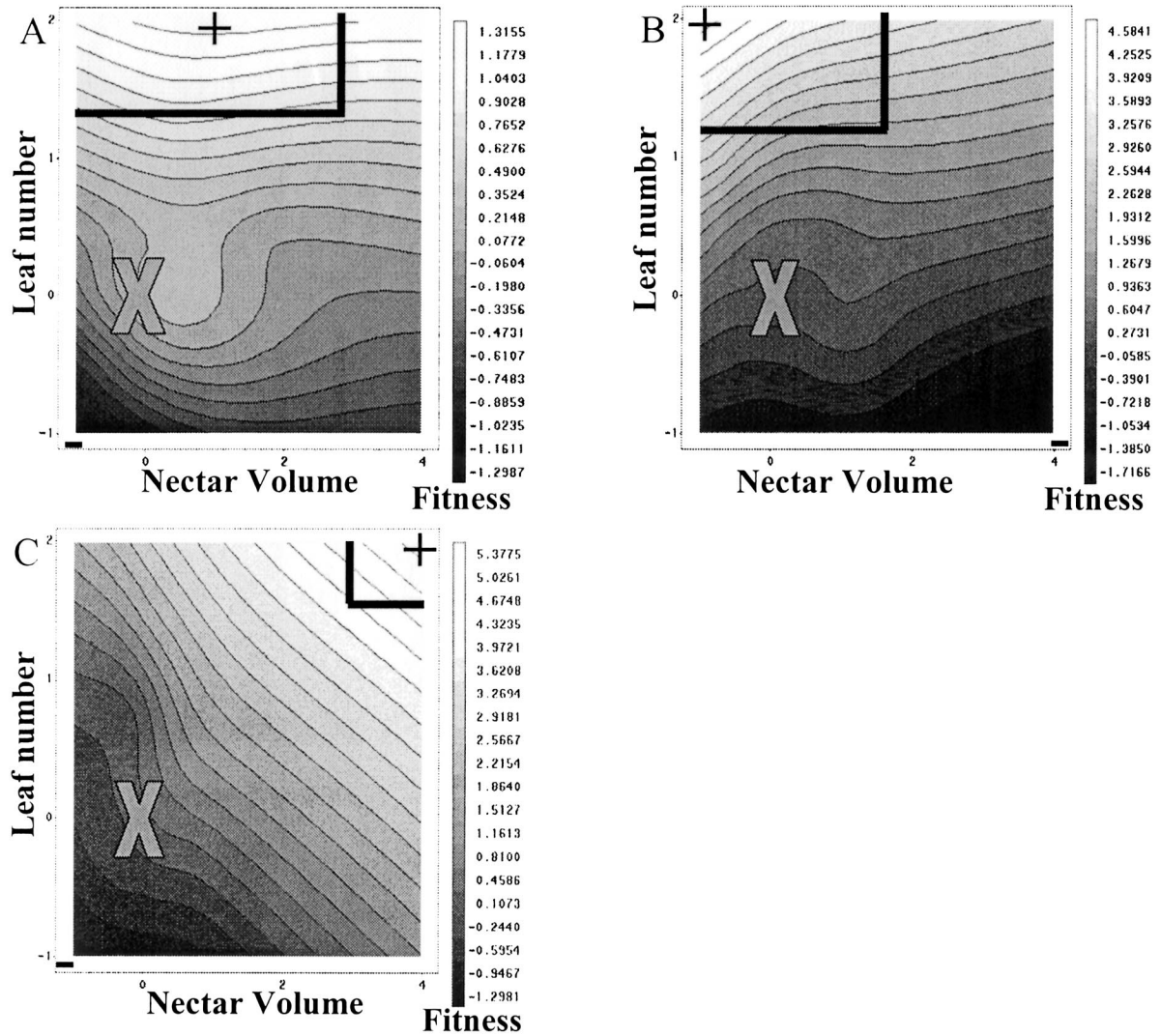


FIG. 1. Selective surfaces of nectar volume per nectary and total leaf number. On all contour plots, relative fitness is on the z-axis, determining height. Darker shading corresponds to lower fitness values, and lighter shading corresponds to higher fitness values. The current mean population value is marked with an X on each surface. The location of fitness peaks are marked with a + in the margin of the figure, and the location of troughs are marked with a -. Traits and fitness are reported as residuals after block effects have been removed. The trait axes are scaled to reflect the total range of family mean values found for each trait. Dark lines denote the 95% confidence interval for the maximum value on the surface, as determined by 10,000 bootstrap replicates. (A) Selective contour for plants sprayed with insecticide. (B) Selective contour for plants without ants. (C) Selective contour for control plants with both ants and other insects.

mean is found (“X” in Fig. 1B), the fitness contours are relatively flat in the direction of the nectar-volume axis. Since the regression analysis has the most power for detecting selection near the population mean where most individuals are, it is not surprising that no directional selection was detected. However, the population mean is also very far from the selectively favored plant size, which corresponds to large plants. At this favored plant size, the extrapolated pattern of selection visualized in the selective surface along the nectar volume axis is steeper than at the current population mean. Thus, there is suggestive evidence for a cost of producing extrafloral nectar, but one that is most pronounced for large plants.

The greater cost of nectar production in the ant-exclusion treatment suggests that any cost of nectar production that was

present in the insecticide treatment may be exacerbated by the additional stress of herbivory. This effect can clearly be seen in the selective pattern imposed by herbivory (Fig. 2A), which is calculated following the technique of Mauricio and Rausher (1997): subtracting the fitness surface of the treatment without insects (Fig. 1A, which represents the input of all selective agents on the plant other than herbivores or ants) from the surface of the treatment with ants (Fig. 1B, which represents the input of all selective agents on the plant other than ants). The resulting fitness surface is essentially a plane that slopes upward from the lower right corner (corresponding to small plants with copious nectar) to the upper left corner (corresponding to large plants with no nectar). The slope of this surface is confirmed by the 95% confidence regions for the maximum and minimum in the lower right

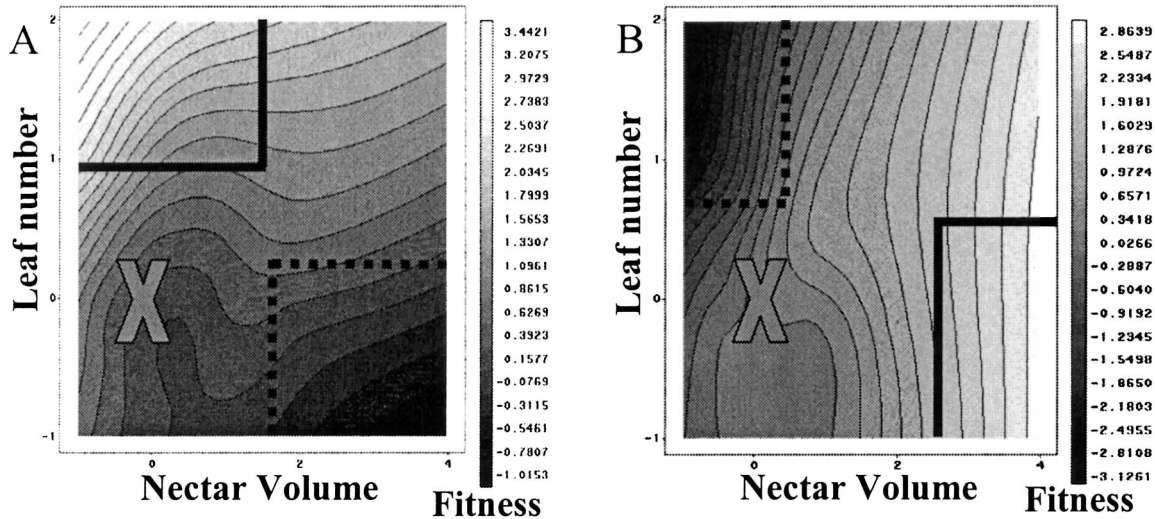


FIG. 2. Selective difference surfaces visualizing the selective effect of including herbivores and ants. As in Figure 1, relative fitness is on the z-axis, determining height. Darker shading corresponds to lower fitness values, and lighter shading corresponds to higher fitness values. The current mean population value is marked with an X on each surface. Traits and fitness are reported as residuals after block effects have been removed. The trait axes are scaled to reflect the total range of family mean values found for each trait. Solid lines indicate a 95% confidence interval for the maximum value of the surface, while dashed lines indicate the 95% confidence interval for the minimum value. (A) Selection on extrafloral nectar volume per nectary and leaf number imposed by herbivores (calculated by subtracting the surface in Fig. 1A from the surface in Fig. 1B). (B) Selection on extrafloral nectar volume per nectary and leaf number imposed by ants (calculated by subtracting the surface in Fig. 1B from the surface in Fig. 1C).

corner and upper left corner, respectively. Thus, for a given plant size, herbivory favors a decrease in the volume of nectar produced per nectary. Because it is unlikely that herbivores are preferentially damaging plants that produce large amounts of nectar, this pattern is most easily explained by assuming that herbivore stress increases the cost of nectar production.

#### *Selection with ants and herbivores present*

Addition of ants further alters the selective surface (control treatment, Fig. 1C). The fitness surface of the control treatment is essentially a plane that slopes upward from the lower left corner (small plants that produce little nectar) to the upper right corner (large plants that produce copious nectar). The confidence region for the fitness peak is small, indicating that both large plants and large nectar volume are favored by selection. Moreover, there is no overlap of the confidence interval of this treatment and that of the ants-excluded treatment, confirming the conclusion from the selection analysis that the addition of ants alters the direction of selection on nectar volume. The form of the plane is also consistent with the detected pattern of correlational selection in that there is a ridge in the left hand side of Figure 1C, indicating a positive interaction between the effects of leaf number and nectar volume on fitness. This pattern suggests that any enhancement of genetic correlations that jointly increase these two traits would be favored by natural selection.

The pattern of selection imposed by ants (Fig. 2B) clearly shows that addition of ants favors increased nectar production and leads to little selection on plant size (the contours are essentially flat in the direction of the leaf number axis). Presumably, this pattern reflects preferential visitation of ants to, and consequent preferential removal of herbivores from, plants producing large amounts of extrafloral nectar.

#### DISCUSSION

The function of extrafloral nectar was a subject of debate for much of the twentieth century, until Bentley (1977) marshaled considerable evidence in support of the notion that the primary function of extrafloral nectar is to attract ‘‘pugnacious’’ bodyguards that protect the plant from herbivores. Until this seminal paper, many authors had argued that extrafloral nectar served physiological functions unrelated to mutualism with insects, such as releasing excess sugars (e.g., Frey-Wyssling 1955; Helder 1958; Mound 1962; Milburn 1975). Research following Bentley’s work has largely supported her conclusions by demonstrating that exclusion of ants from plants with extrafloral nectaries, including *Chamaecrista fasciculata* (Barton 1986; Kelly 1986), frequently results in increased damage by herbivores and decreased mean plant survival and/or reproductive success (e.g. Oliveira 1997; Oliveira et al. 1999; Cuaulte and Rico-Gray 2003). However, because most of this work has not explicitly examined the effects of variation in nectar characteristics, and none has explicitly examined patterns of selection on such characters in the absence of ants, several issues regarding the evolution of extrafloral nectar production have remained unresolved. Our experiment provides some resolution to these issues.

First, although a decrease in fitness associated with ant removal (as found in previous studies) indicates that there is a benefit to attracting ants by producing nectar, it does not confirm that ants are involved in the maintenance of extrafloral nectar characters. More specifically, it does not demonstrate that attracting ants is a crucial benefit, such that removal of ants would result in selection acting to eliminate extrafloral nectar production. However, our experiment indicates that ant attraction is such a crucial benefit of nectar

production in *Chamaecrista fasciculata*. In this species, in the presence of ants, high levels of nectar production are favored by selection, but in the absence of ants there is little direct selection and the selective surfaces suggest lower nectar volumes per nectary would eventually be favored. This result does not necessarily mean that other benefits associated with nectar production are absent, but does demonstrate that these benefits by themselves do not generate a net pattern of selection that would maintain nectar production.

Second, although previous investigations have demonstrated benefits associated with ant visitation, they have not attempted to determine whether extrafloral nectar production has costs, nor whether the benefits of nectar production exceed the costs. In our insecticide treatment, which excluded both ants and herbivores, there was little evidence of costs associated with nectar production, in terms of the amount of nectar produced per nectary. However, this treatment is somewhat artificial because although ants may not be present in some natural populations, our observations suggest that herbivores normally are. Under these more natural conditions, corresponding to our ant-exclusion treatment, we found suggestive evidence of costs of nectar production. Moreover, in the presence of ants there was net selection favoring high nectar levels, indicating that benefits of nectar production exceed the costs.

Finally, whereas most previous investigations have suggested that plant fitness differences are associated with presence versus absence of ants, and thus by inference with the presence versus absence of extrafloral nectar production, these studies do not indicate what aspects of nectar production are subject to selection by ants. Studies of ant responses to variation in several nectar characteristics, including nectar volume, and sugar and amino-acid concentrations (Lanza 1988, 1991; Lanza et al. 1993; Koptur and Truong 1998; Kay 2002), suggest that any of these continuous characteristics might be molded by selection imposed by ants. One recent study of extrafloral nectar in wild cotton by Rudgers (2004) provides a strong example of the potential of selection to act explicitly on extrafloral nectar production. Rudgers simultaneously examined the effect of excluding ants and the effect of blocking all extrafloral nectar production. This study found that the benefits of ant protection were greatly reduced when extrafloral nectar production was completely suppressed. Our study confirms that continuous variation in nectar volume, is subject to selection by ants and thus can be expected to vary among populations that differ in ant availability.

#### *Applicability of Cost-Benefit Models*

Although many theoretical analyses of the evolution of mutualisms incorporate cost-benefit analyses either explicitly or implicitly (see introduction), empirical analyses of mutualisms have seldom explicitly examined the appropriateness of a cost-benefit framework, except in the case of plant-pollinator interactions (e.g., Holland and Fleming 1999). Because our experimental manipulations allow us to dissect component costs and benefits associated with extrafloral nectar production, we are able to determine the applicability of such a framework to mutualisms between ants and plants. Specifically, we can evaluate the appropriateness of three

assumptions that would characterize any cost-benefit analysis of mutualisms:

*Extrafloral nectar is costly to provide.*—Although there is little direct evidence for costs of extrafloral nectar production, several types of indirect evidence suggest that nectar costs may be substantial. For example, one study has demonstrated a fitness cost associated with producing floral nectar (Pyke 1991). Because the basic components of floral and extrafloral nectar are similar (Baker et al. 1978; Koptur 1994), this observation suggests that similar costs are likely to be associated with extrafloral nectar. Another type of suggestive evidence is the inducibility of extrafloral nectar production in response to herbivory (Koptur 1989; Smith, et al. 1990; Heil et al. 2001; Mondor and Addicott 2003; Ness 2003; Wäckers and Bonifay 2004). Such inducibility has been interpreted as evidence of costs in chemical plant defenses (Simms 1992). An analogous interpretation points to a cost of extrafloral nectar production (Agrawal and Rutter 1998; Rudgers and Gardener 2004).

As described above, our results provide suggestive evidence that extrafloral nectar production in *C. fasciculata* could be costly. Although for the ants-excluded treatment we detected no negative genetic correlation between fitness and nectar volume in our selection analysis, this result is likely due to the relative flatness of the fitness surface at the population mean. Thus for the extant population, nectar production is selectively neutral when ants are not present. The population mean, however, is far away from the peak of the fitness surface, indicating that the population is not at equilibrium with respect to plant size. This disparity between the fitness peak and the current population mean could reflect changing selection pressures or genetic constraints stemming from trade-offs between total leaf number and other traits that we did not measure in our study. If the population could reach the equilibrium point in our extrapolated fitness surfaces, where plant size would be large, there would be decreasing fitness with increasing nectar volume, indicating a cost to nectar production. As noted previously, this cost is not detectable in the absence of herbivory. However, because most populations of *C. fasciculata* experience herbivory, costs would most likely be expressed in natural populations with high leaf numbers.

*Extrafloral nectar production yields a benefit.*—Demonstrating whether or not extrafloral nectar yields a benefit to plants is the traditional preoccupation of studies of ant-plant mutualisms mediated by extrafloral nectar production (e.g., Janzen 1966; Elias and Gelband 1975; Bentley 1977; Keeler 1977, 1981; Tilman 1978; Koptur 1979; O'Dowd 1979; Stephenson 1982; Horvitz and Schemske 1984; Barton 1986; Kelly 1986; Smiley 1986; Rashbrook et al. 1992; Koptur et al. 1998; Oliveira et al. 1999; Freitas et al. 2000; Ruhren 2003; Cuaulte and Rico-Gray 2003). The core of this approach involves observing ants feeding on extrafloral nectar, removing ants from some plants, and discerning if plants with ants have a higher fitness than those that do not. This type of experiment can demonstrate whether there is a benefit associated with the presence versus absence of nectaries but provides little information regarding the relative benefits of different values of quantitative nectary traits. In the context of this study, this type of analysis cannot reveal whether the

benefit associated with nectar production increases as nectar volume increases, or whether any such increase in benefit plateaus once enough ants have been attracted to remove all herbivores.

Addressing these issues requires experimental dissection of the overall pattern of selection on nectar volume into components corresponding to benefits and costs. In this study, we have been able to achieve such a dissection. Our results demonstrate that the benefit of nectar production, which corresponds to the pattern of selection imposed by ants (Fig. 2B), increases monotonically, indicating that greater nectar volume per nectary yields a higher benefit. There is some indication that the rate of this increase diminishes as nectar volume increases: the fitness contours in Figure 2B become slightly more widely spaced as nectar volume increases. However, there is little evidence of a plateau in benefit, suggesting that even if nectar volume could be increased beyond the range of values observed in this study, additional benefit would accrue. Finally, there is positive correlational selection between leaf number and nectar volume per nectary, suggesting that when ants are present, selection favors genetic connections between producing high levels of nectar and producing many leaves. Correlational selection is frequently the result of trade-offs (Sinervo and Svensson 2002), and the action of selection to favor plants with many leaves and high nectar volume per nectary and disfavor plants with few leaves and low nectar production may be the result of a trade-off involving costs of nectar production. Furthermore, given that increased leaf number was associated with increased herbivory, plants with high leaf number may derive the most benefit from ant protectors.

*The costs and benefits of extrafloral nectar production yield an intermediate optimum maintained by stabilizing selection.*—One predicted outcome of many cost-benefit models is that the trait in question equilibrates at an intermediate value and is thereafter subject to stabilizing selection (Roughgarden 1975; Keeler 1985; Connor 1995; Schwartz and Hoeksema 1998). The existence of costs and benefits does not by itself guarantee such an intermediate optimum, which normally requires that one or both of the cost and benefit functions be nonlinear. Our experiment failed to reveal the existence of such an optimum for nectar volume. Thus, it appears that in this system costs and benefits do not yield an intermediate optimum, at least within the range of variation encompassed in our experiment. Such an optimum may exist, but outside the range of variation we observed. Alternatively, costs and benefits themselves may be temporally and/or spatially quite variable if, for example, the abundance or species composition of ants varies. Under such variability, there may be no sites or times at which an intermediate optimum exists. A major caveat of our study is that it represents a measurement of the patterns of selection in only a single season, and longer-term patterns of evolution of extrafloral nectar production may be shaped by selective pressures not observed during this year.

Our analysis has shown that application of a cost-benefit framework yields some insights into the evolution of a quantitative mutualism trait. We have obtained evidence for the existence of both costs and benefits associated with that trait and quantified the relationship between the magnitudes of

costs and benefits and the value of the trait. We have also demonstrated that, at least for a single population at one site in one year, benefits exceed costs and thus selection favors retention of that trait. However, application of a cost-benefit framework to this dataset provides little insight into the evolutionary equilibrium of the trait. The interaction of costs and benefits does not produce an evolutionary optimum for the trait within the range of variation observed, and the current population mean for the trait appears to be far from any such optimum. These results suggest that successful application of a cost-benefit framework to extrafloral nectar production will require incorporation of the influence of temporal and spatial variation on the form and magnitude of costs and benefits (Rudgers and Gardener 2004). To observe stabilizing selection, it may be necessary to further expand the range of variation in the population through artificial selection.

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#### LITERATURE CITED

- Agrawal, A. A., and M. T. Rutter. 1998. Dynamic anti-herbivore defense in ant-plants: the role of induced responses. *Oikos* 83: 227–236.
- Allison, P. D. 1999. Logistic regression using the SAS system: theory and application. SAS Institute, Inc., Cary, NC.
- Baker, H. B., P. A. Opler, and I. Baker. 1978. A comparison of the amino acid complements of floral and extrafloral nectars. *Bot. Gaz.* 139:322–332.
- Barton, A. M. 1986. Spatial variation in the effect of ants on an extrafloral nectary plant. *Ecology* 67:495–504.
- Bentley, B. L. 1977. Extrafloral nectaries and protection by pug-nacious bodyguards. *Annu. Rev. Ecol. Syst.* 8:408–427.
- Bergstrom, C. T., and M. Lachman. 2003. The Red King effect: when the slowest runner wins the coevolutionary race. *Proc. Natl. Acad. Sci. USA* 100:593–598.
- Bronstein, J. L. 1994a. Our current understanding of mutualism. *Q. Rev. Biol.* 69:31–51.
- . 1994b. Conditional outcomes in mutualistic interactions. *Trends Ecol. Evol.* 9:214–217.
- . 1998. The contribution of ant plant protection studies to our understanding of mutualism. *Biotropica* 30:150–161.
- Campbell, D. R. 1996. Evolution of floral traits in a hermaphroditic plant: field measurements of heritabilities and genetic correlations. *Evolution* 50:1442–1453.
- Caruso, C. M. 2001. Differential selection on floral traits in *Ipomopsis aggregata* growing in contrasting environments. *Oikos* 94: 295–302.
- Conner, J. K., S. Rush, and P. Jennetten. 1996. Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). I. Selection through lifetime female fitness. *Evolution* 50:1127–1136.
- Connor, R. C. 1995. The benefits of mutualism: a conceptual framework. *Biol. Rev.* 70:427–457.
- Cuaulte, M., and V. Rico-Gray. 2003. The effect of wasps and ants on the reproductive success of the extrafloral nectaried plant *Turnera ulmifolia* (Turneraceae). *Funct. Ecol.* 17:417–423.
- Doebeli, M., and N. Knowlton. 1998. The evolution of interspecific mutualisms. *Proc. Natl. Acad. Sci. USA* 95:8676–8680.

- Elias, T. S. 1983. Extrafloral nectaries: their structure and distribution. Pp. 174–203 in B. Bentley and T. Elias, eds. *The biology of nectaries*. Columbia Univ. Press, New York.
- Elias, T. S., and H. Gelband. 1975. Nectar: its production and function in trumpet creeper. *Science* 189:289–291.
- Fenster, C. B. 1991. Gene flow in *Chamaecrista fasciculata* (Leguminosae). I. Gene dispersal. *Evolution* 45:398–409.
- Ferdy, J., L. Despres, and B. Godelle. 2002. Evolution of mutualism between globe-flowers and their pollinating flies. *J. Evol. Biol.* 217:219–234.
- Freitas, L., L. Galetto, G. Bernardello, and A. A. S. Paoli. 2000. Ant exclusion and reproduction of *Croton sacropetalus* (Euphorbiaceae). *Flora* 195:398–402.
- Frey-Wyssling, A. 1955. The phloem supply to the nectaries. *Acta Bot. Neerl.* 4:358–369.
- Futuyma, D. J. 1998. *Evolutionary biology*. 3rd ed. Sinauer, Sunderland, MA.
- Gilbert, L. E. and P. H. Raven, eds. 1975. *Coevolution of animals and plants*. Univ. of Texas Press, Austin.
- Heil, M., T. Koch, A. Hilpert, B. Fiala, W. Boland, and K. E. Linsenmair. 2001. Extrafloral nectar production of the ant-associated plant, *Macaranga tanarius*, is an induced, indirect, defensive response elicited by jasmonic acid. *Proc. Natl. Acad. Sci. USA* 93:1083–1088.
- Helder, R. J. 1958. The excretion of carbohydrates (nectaries). *Handbuch der Pflanzenphysiologie* 6:978–990.
- Herrera, C. M. 1993. Selection on floral morphology and environmental determinants of fecundity in a hawkmoth pollinated violet. *Ecol. Monogr.* 63:251–275.
- Holland, J. N., and T. H. Fleming. 1999. Mutualistic interactions between *Upiga virescens* (Pyralidae), a pollinating seed consumer, and *Lophocereus schottii* (Cactaceae). *Ecology* 80:2074–2084.
- Horvitz, C. C., and D. W. Schemske. 1984. Effects of ants and an ant-tended herbivore on seed production of a neotropical herb. *Ecology* 65:1369–1378.
- Janzen, D. H. 1966. Coevolution of mutualism between ants and acacias in Central America. *Evolution* 20:249–275.
- . 1985. The natural history of mutualism. Pp. 40–99 in D.H. Boucher, ed. *The biology of mutualism: ecology and evolution*, Oxford Univ. Press, New York.
- Juenger, T., and J. Bergelson. 1998. Pairwise versus diffuse natural selection and the multiple herbivores of scarlet gilia, *Ipomopsis aggregata*. *Evolution* 52:1583–1592.
- Kay, A. 2002. Applying optimal foraging theory to assess nutrient availability ratios for ants. *Ecology* 83:1935–1944.
- Kearns, C. A., and D. W. Inouye. 1993. *Techniques for pollination biologists*. Univ. Press of Colorado, Niwot, Colorado.
- Keeler, K. H. 1977. Extra-floral nectaries of *Ipomoea carnea* (Convolvulaceae). *Am. J. Bot.* 64:1182–1188.
- . 1981. Function of *Mentzelia nuda* (Loasaceae) post-floral nectaries in seed defense. *Am. J. Bot.* 68:295–299.
- . 1985. Cost:benefit models of mutualism. Pp. 100–127 in D.H. Boucher, ed. *The biology of mutualism: ecology and evolution*, Oxford Univ. Press, New York.
- . 1989. Ant-plant interactions. Pp. 207–242 in W.G. Abrahamson, ed. *Plant-animal interactions*. McGraw-Hill Book Co., New York.
- Kelly, C. A. 1986. Extrafloral nectaries: ants, herbivores, and fecundity in *Cassia fasciculata*. *Oecologia* 69:600–605.
- Koptur, S. 1979. Facultative mutualism between weedy vetches bearing extrafloral nectaries and weedy ants in California. *Am. J. Bot.* 66:1016–1020.
- . 1989. Is extrafloral nectar an inducible defense? Pp. 323–339 in J. Bock and Y. Linhart, eds. *Evolutionary ecology of plants*. Westview Press, Boulder, CO.
- . 1994. Floral and extrafloral nectars of Costa Rican Inga trees: a comparison of their constituents and composition. *Biotropica* 26:276–284.
- Koptur, S., and N. Truong. 1998. Facultative ant-plant interactions: nectar sugar preferences of introduced pest ant species in south Florida. *Biotropica* 30:179–189.
- Koptur, S., V. Rico-Gray, and M. Palacios-Rios. 1998. Ant protection of the nectaried fern *Polypodium plebeium* in central Mexico. *Am. J. Bot.* 85:736–739.
- Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution* 37:1210–1226.
- Lanza, J. 1988. Ant preference for *Passiflora* nectar mimics that contain amino acids. *Biotropica* 20:341–344.
- . 1991. Response of fire ants (Formicidae, *Solenopsis invicta* and *S. geminata*) to artificial nectars with amino acids. *Ecol. Entomol.* 16:203–210.
- Lanza, J., E. Vargo, S. Pulim, and Y. Z. Chang. 1993. Preferences of the fire ants *Solenopsis invicta* and *S. geminata* (Hymenoptera: Formicidae) for amino acid and sugar components of extrafloral nectaries. *Environ. Entomol.* 22:411–417.
- Lee, T. D., and F. A. Bazzaz. 1982. Regulation of fruit and seed production in an annual legume, *Cassia fasciculata*. *Ecology* 63:1374–1388.
- Leiss, K. A., K. Vrieling, and P. G. L. Klinkhamer. 2004. Heritability of nectar production in *Echium vulgare*. *Heredity* 92:446–451.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. *SAS system for mixed models*. SAS Institute, Inc., Cary, NC.
- Lynch, M., and J. B. Walsh. 1998. *Genetics and analysis of quantitative traits*. Sinauer Sunderland, MA.
- Mauricio, R., and M. D. Rausher. 1997. Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution* 51:1435–1444.
- Milburn, J. A. 1975. Pressure flow. Pp. 328–353 in M. H. Zimmerman and J. A. Milburn, eds. *Encyclopedia of plant physiology: transport in plants. I. Phloem Transport*. Springer-Verlag, New York.
- Mitchell, R. J. 2004. Heritability of nectar traits: why do we know so little? *Ecology* 85:1527–1533.
- Mitchell-Olds, T., and R. G. Shaw. 1987. Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* 41:1149–1161.
- Mondor, E. B. and J. F. Addicott. 2003. Conspicuous extra-floral nectaries are inducible in *Vicia faba*. *Ecol. Lett.* 6:495–497.
- Mound, L. A. 1962. Extrafloral nectaries of cotton and their secretions. *Emp. Cotton Grow. Rev.* 39:254–261.
- Ness, J. H. 2003. *Catalpa bignonioides* alters extrafloral nectar production after herbivory and attracts ant bodyguards. *Oecologia* 134:210–218.
- O'Dowd, D. J. 1979. Foliar nectar production and ant activity on a Neotropical tree, *Ochroma pyramidale*. *Oecologia* 43:233–248.
- O'Dowd, D. J., and E. A. Catchpole. 1983. Ants and extrafloral nectaries: no evidence for plant protection in *Helichrysum* spp.-ant interactions. *Oecologia* 59:191–200.
- Oliveira, P. 1997. The ecological function of extrafloral nectaries: herbivore deterrence by visiting ants and reproductive output in *Caryocar brasiliense* (Caryocaraceae). *Funct. Ecol.* 11:323–330.
- Oliveira, P., V. Rico-Gray, C. Diaz-Castelazo, and C. Castillo-Guevara. 1999. Interaction between ants, extrafloral nectaries, and insect herbivores in Neotropical sand dunes: herbivore deterrence by visiting ants increases fruit set in *Opuntia stricta* (Cactaceae). *Funct. Ecol.* 13:623–631.
- Pemberton, R. W., and J. H. Lee. 1996. The influence of extrafloral nectaries on parasitism of an insect herbivore. *Am. J. Bot.* 83:1187–1194.
- Pierce, N. E., R. L. Kitching, R. C. Buckley, M. F. J. Taylor, and K. Benbow. 1987. Costs and benefits of cooperation between the Australian lycaenid butterfly, *Jalmenus evagoras* and its attending ants. *Behav. Ecol. Sociobiol.* 21:237–248.
- Pyke, G. H. 1991. What does it cost a plant to produce floral nectar? *Nature* 350:58–59.
- Rashbrook, V. K., S. G. Compton, and J. H. Lawton. 1992. Ant-herbivore interactions: reasons for the absence of benefits to a fern with foliar nectaries. *Ecology* 73:2167–2174.
- Rausher, M. D. 1992. The measurement of selection of quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* 46:616–626.

- Roff, D. A. 2001. The threshold model as a general purpose normalizing transformation. *Heredity* 86:404–411.
- Roughgarden, J. 1975. Evolution of a marine symbiosis: a simple cost-benefit model. *Ecology* 56:1201–1208.
- Rudgers, J. A. 2004. Enemies of herbivores can shape plant traits: selection in a facultative ant-plant mutualism. *Ecology* 85:192–205.
- Rudgers, J. A., and M. C. Gardener. 2004. Extrafloral nectar as a resource mediating multispecies interactions. *Ecology* 85:1495–1502.
- Ruhren, S. 2003. Seed predators are undeterred by nectar-feeding ants on *Chamaecrista nictitans* (Caesalpineaceae). *Plant Ecol.* 166:189–198.
- SAS Institute. 2000. SAS/STAT user's guide, Vers. 8. SAS Institute, Cary, NC.
- Schemske, D. W., and C. C. Horvitz. 1989. Temporal variation in selection on a floral character of a neotropical herb. *Evolution* 43:461–464.
- Schluter, D., and M. L. Nychka. 1994. Exploring fitness surfaces. *Am. Nat.* 143:597–616.
- Schwartz, M. W., and J. D. Hoeksema. 1998. Specialization and resource trade: biological markets as a model of mutualisms. *Ecology* 79:1029–1038.
- Self, S. G., and K. Y. Liang. 1987. Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under non-standard conditions. *J. Am. Stat. Assoc.* 82:605–610.
- Simms, E. L. 1992. Costs of plant resistance to herbivores. Pp. 392–425 in R. S. Fritz and E. L. Simms, eds. *Plant resistance to herbivores and pathogens. Ecology, evolution, and genetics.* Univ. of Chicago Press, Chicago, IL.
- Sinervo, B., and E. Svensson. 2002. Correlational selection and the evolution of genomic architecture. *Heredity* 89:329–338.
- Smiley, J. 1986. Ant constancy at *Passiflora* extrafloral nectaries: effects on caterpillar survival. *Ecology* 67:516–521.
- Smith, L. L., J. Lanza, and G. C. Smith. 1990. Amino acid concentrations in extrafloral nectar of *Impatiens sultani* increase after simulated herbivory. *Ecology* 71:107–115.
- Stapel, J. O., A. M. Cortesero, C. M. DeMoraes, J. H. Tumlinson, and W. J. Lewis. 1997. Extrafloral nectar, honeydew, and sucrose effects on searching behavior and efficiency of *Microplitis croceipes* (Hymenoptera: Braconidae) in cotton. *Environ. Entomol.* 26:617–623.
- Stapel, J. O., A. M. Cortesero, and W. J. Lewis. 2000. Disruptive sublethal effects of insecticides on biological control: altered foraging ability and lifespan of a parasitoid after feeding on extrafloral nectar of cotton treated with systemic insecticides. *Biol. Control* 17:243–249.
- Stephenson, A. G. 1982. The role of the extrafloral nectaries of *Catalpa speciosa* in limiting herbivory and increasing fruit production. *Ecology* 63:663–669.
- Stinchcombe, J. R., and M. D. Rausher. 2001. Diffuse selection on resistance to deer herbivory in the ivyleaf morning glory, *Ipomoea hederacea*. *Am. Nat.* 158:376–388.
- Stinchcombe, J. R., M. T. Rutter, D. S. Burdick, P. L. Tiffin, M. D. Rausher, and R. Mauricio. 2002. Testing for environmentally induced bias in phenotypic estimates of natural selection: theory and practice. *Am. Nat.* 160:511–523.
- Tempel, A. S. 1983. Bracken fern (*Pteridium aquilinum*) and nectar-feeding ants: a nonmutualistic interaction. *Ecology* 64:1411–1422.
- Tiffin, P., and M. D. Rausher. 1999. Genetic constraints and selection acting on tolerance to herbivory in the common morning glory, *Ipomoea purpurea*. *Am. Nat.* 154:700–716.
- Tilman, D. 1978. Cherries, ants, and tent caterpillars: timing of nectar production in relation to susceptibility of caterpillars to ant predation. *Ecology* 59:686–692.
- Wäckers, F. L., and C. Bonifay. 2004. How to be sweet? Extrafloral nectar allocation by *Gossypium hirsutum* fits optimal defense theory predictions. *Ecology* 85:1512–1518.
- West, S. A., E. T. Kiers, E. L. Simms, and R. F. Denison. 2002. Sanctions and mutualism stability: why do rhizobia fix nitrogen? *Proc. R. Soc. Lond. B* 269:685–694.
- Wyatt, R., S. B. Broyles, and G. S. Derda. 1992. Environmental influences on nectar production in milkweeds (*Asclepias syriaca* and *A. exaltata*). *Am. J. Bot.* 79:636–642.

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