Adaptive Divergence in a Defense Symbiosis
Driven from the Top Down

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ABSTRACT: Most studies of adaptive radiation in animals focus on resource competition as the primary driver of trait divergence. The roles of other ecological interactions in shaping divergent phenotypes during such radiations have received less attention. We evaluate natural enemies as primary agents of diversifying selection on the phenotypes of an actively diverging lineage of gall midges on tall goldenrod. In this system, the gall of the midge consists of a biotrophic fungal symbiont that develops on host-plant leaves and forms distinctly variable protective carapaces over midge larvae. Through field studies, we show that fungal gall morphology, which is induced by midges (i.e., it is an extended phenotype), is under directional and diversifying selection by parasitoid enemies. Overall, natural enemies disruptively select for either small or large galls, mainly along the axis of gall thickness. These results imply that predators are driving the evolution of phenotypic diversity in symbiotic defense traits in this system and that divergence in defensive morphology may provide ecological opportunities that help to fuel the adaptive radiation of this genus of midges on goldenrods. This enemy-driven phenotypic divergence in a diversifying lineage illustrates the potential importance of consumer-resource and symbiotic species interactions in adaptive radiation.

Keywords: multitrophic interactions, directional, diversifying, stabilizing selection, Cecidomyiidae, Asteromyia carbonifera.

Introduction

Natural enemies (predators, parasites, and pathogens) have long been thought to be important causes of divergent selection, character shifts in phenotypic traits, and ultimately evolutionary diversification (e.g., apostatic selection, reflexive selection, and aspect diversity; Cain and Sheppard 1954; Fisher 1958; Moment 1962; Clarke 1969; Kettlewell 1973; Paulson 1973; Ricklefs and O’Rourke 1975). But empirical support for an important role of predation in comparison to other selective causes of phenotypic divergence has lagged behind that of competition, resource heterogeneity, and reproduction (Langerhans 2006; Svensson and Friberg 2007). Predators are obviously important in the evolution of prey defenses, including morphology, camouflage, chemical defenses, or behavior. Predators can also select for variation in adaptive phenotypes within and between prey species; for example, when semi-isolated prey populations are subject to different predator regimes or when apparent competition via shared enemies selects for ecological trait divergence (Holt 1977; Abrams 2000; Schluter 2000; Langerhans et al. 2007). Predation can also fuel adaptive radiation if escape from predation corresponds to the emergence of new ecological opportunities, niche shifts, or isolating mechanisms acting to reduce gene flow between ancestral and derived lineages (Singer and Stireman 2005; Rueffler et al. 2006; Nosil 2012).

Previous approaches to understanding how predation shapes diversification have coupled experimental and phylogenetic approaches in groups undergoing adaptive radiations where variation in defense-related traits is evident (Vamosi and Schluter 2004; Vamosi 2005; Nosil and Crespi 2006). In such groups, one expectation is that if a history of predation has been important in promoting the adaptive radiation of a prey species, there should be evidence of selection on traits related to defense (Abrams 2000; Langerhans et al. 2007; Marchinko 2009). In a few cases, divergent selection on defense traits associated with alternative predator regimes has been demonstrated in natural populations, most unambiguously in groups where substantial prior evidence indicates that adaptive divergence is underway, such as Gambusia fish (Langerhans et al. 2007), sticklebacks (Marchinko 2009), and crossbills (Parchman and Benkman 2002). As well, in recent years there has been a growing interest in broadening the scope of species interactions in biological diversification even further by accounting for how symbionts and mutualists...
shape diversification (Brucker and Bordenstein 2012), particularly those that confer defense against predators (Oliver et al. 2014).

Using a defense symbiosis between a fly and a fungus, we evaluate and quantify the selective role that predation plays in driving the evolution of adaptive phenotypes. Asteromyia gall midges exhibit all of the hallmarks of adaptive radiation, that is, rapid diversification of a lineage with corresponding divergence of adaptive phenotypes (Schluter 2000). This rapid radiation across goldenrods (Solidago spp.) in North America is accompanied by the accumulation of divergent phenotypes in the shape of their fungal symbiont (Stireman et al. 2008, 2010, 2012). The fungal symbiont protects gall midge larvae from attack by parasitoid wasps, and our primary objective is to evaluate patterns of selection on defense-related traits (i.e., gall thickness and diameter) among populations of the species Asteromyia carbonifera on their common host plant Solidago altissima. We show that top-down pressures consisting of predators that may specialize on alternative phenotypes is currently spurring phenotypic divergence and likely has contributed to a remarkable adaptive radiation of this defense-based symbiosis.

Material and Methods

Study System

The Cecidomyiidae gall midge Asteromyia carbonifera (O.S.) is found on North American goldenrods (Solidago spp.), where it engages in a symbiotic interaction with an ascomycete fungus (Botryosphaeria dothidea; Botryosphaeriaceae, Botryosphaeriales) that forms a nutritive and protective carpophore over the midge larvae (Borkent and Bissett 1985; Bissett and Borkent 1988; Crego et al. 1990; Heath and Stireman 2010; Janson et al. 2010). The adult female flies vector the fungus to goldenrod leaves, where larvae influence the growth and development of the fungus into galls of various morphotypes (hereafter morphs; fig. 1). In A. carbonifera populations on tall goldenrod (Solidago altissima L.), galls can be divided into four characteristic morphs that differ in thick-
ness, diameter, and number of larval chambers. Previous researchers (Crego et al. 1990) have named these crescent, cushion, flat, and irregular morphs (fig. 1). The fungus itself is isogenic between morphs (Janson et al. 2010), and it is the midge lineages that are genetically distinct rather than the fungus. The midge larvae use the fungus as their main food source as well as for protection from environmental threats (Janson et al. 2009; Heath and Stireman 2010). Cushion and irregular morphs have been found on only S. altissima and most likely diverged there. All gall morphs appear to be broadly distributed across the extensive range of S. altissima in North America, and they often co-occur on the same plant ramet and even the same leaves, suggesting that gall morph is not strongly affected by host plants (Crego et al. 1990; Stireman et al. 2008). This is consistent with broader phylogenetic patterns in Asteromyia that suggest multiple origins of fungal gall morphs within different host plant species, superimposed on host plant–associated differentiation (Stireman et al. 2012). However, it is possible that fungal growth and thus gall size is influenced by plant genotype, as has been found in studies of direct plant gall formers (Price and Clancy 1986; Weis and Abrahamson 1986). Multiple species of parasitoid wasps attack the encased larva by piercing the fungal galls with their needle-like ovipositors, and mortality rates can locally exceed 90% (Weis 1982a, 1982b, 1983; Weis et al. 1983). Collectively, the galls are attacked by up to seven different parasitoid wasps with different oviposition and life-history requirements, including Aprostocetus sp. (Hymenoptera: Eulophidae), Aprostocetus tesserus (Burkes) (Hymenoptera: Eulophidae), Torymus capite (Huber) (Hymenoptera: Torymidae), Platygaster solidaginis (Ashmed) (Hymenoptera: Platygasteridae), Aprostocetus homeri (Girault) (Hymenoptera: Eulophidae), Baryscapus fumipennis (Girault) (Hymenoptera: Eulophidae), and Closterocerus solidaginis (Yoshimoto) (Hymenoptera: Eulophidae). Previous work has revealed no evidence that midge galls compete for resources (Heath et al. 2014). However, it may be that the different morphs map to distinct specificities in parasitoid wasps and that some wasps are more successful in attacking certain shapes over others (Stireman et al. 2010, 2012). Furthermore, wasp attack on common fungal morphs may favor extreme phenotypes through apparent competition, resulting in divergent (disruptive and/or directional) selection on fungal morphs. Such divergent top-down selection on gall morphology combined with repeated shifts among host plant species may underlie the explosive diversification of Asteromyia midge lineages on goldenrods (Stireman et al. 2012; Mullen and Shaw 2014).

We used common garden experiments of S. altissima plants to address the following questions regarding the function of gall morphology and the potential role of enemies in divergence of A. carbonifera morphotypes: (1) How and to what degree are galls morphologically discrete? (2) How does plant genotype affect gall morphology? (3) Do parasitoid taxa vary in their use of gall morphs? (4) What is the shape and strength of selection by parasitoids on gall morphology?

### Common Garden of Goldenrod Accessions

We established a common garden of S. altissima accessions in June 2008 on the campus of Wright State University (39.7876°N, 84.0528°W, 256 m). Plants were grown in the greenhouse in spring from rhizomes collected haphazardly from nearby wild populations (Heath et al. 2014). We transplanted the plants to the field in a 10 × 10 grid spaced 2 m on center in a randomized complete block design; each 2 × 10 block contained two replicates of each of 10 clones. We watered the plants after transplanting, but other than periodical weeding and mowing around the replicates, they were left to establish on their own. In a previous study during the summer of 2009 and the fall of 2010, we measured various parameters (e.g., height, leaf morphology) for each of the replicates (n = 10 per accession) to assess phenotypic and genotypic variation in the 10 accessions. Ordination of these parameters illustrated that all but two of these accessions were morphologically distinguishable (see Heath et al. 2014). Therefore, the 10 accessions represent at least nine genotypes of S. altissima, with accession 11.1 and 11.12 likely representing closely related genotypes.

### Tracking Gall Development

Starting in May 2009, we surveyed the common garden subplots daily for new galls, which were marked and measured at a rate of about one to two rows of the common garden per day. After about 1–2 weeks, a complete census of the galls in the garden was achieved, and the process began again, including additional measurements of the previously marked galls and measurements of any newly occurring galls. This was continued until August, at which time more than 2,000 galls had been tracked and measured (crescents, n = 1,579; irregulars, n = 210; cushions, n = 307; flats, n = 188). The same process was continued in 2010, which yielded data for 6,733 galls (crescents, n = 1,591; irregulars, n = 2,167; cushions, n = 1,079; flats, n = 903; unknown = 993). We relied on natural colonization of the garden by surrounding A. carbonifera populations, and the increasing numbers and varying ratios of morphs across years may reflect colonization dynamics and variation in dispersal among populations in addition to local population processes. Most unknown galls were not completely measured and evaluated for parasitism because of time constraints, but data on diameter existed from tracking them to maturity in the field, and therefore they were retained in the 2010 database. Morph assignment was confirmed visually after the marked galls were
mature (i.e., when the rate of gall diameter increase was <10% per week), at which time the leaf they were on was collected. The collected galls were stored at 4°C for at most a few days before being evaluated for parasitism and measured.

Assessing Parasitism and Trait Measurement
The stored galls were either reared in individual cotton-stopped vials in plastic chambers (moist peat moss below a perforated Plexiglas tray) or dissected. Because the galls were collected when they were mature, most of the parasitoids and gall midges were in the pupal stage, which resulted in high rearing success and straightforward identification. We measured the thickness and maximum diameter of mature galls with digital calipers to 0.01 mm. After 2 weeks in the rearing chambers, galls were dissected and assessed for the number and presence of seven different parasitoid species and the number of gall midge larval chambers. The identity of representative parasitoid specimens collected in 2009 was confirmed by two experts (J. La Salle, Commonwealth Scientific and Industrial Research Organization; C. Hannson, Lund University). Only the 2010 data set was used in this study, and it is provided in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.p49b1 (Heath et al. 2018).

Kernel Density Contour Plots
To visualize the distribution of gall morphology by gall morphotype, a kernel density contour plot of standardized gall diameter and thickness was generated with the kde2d function in the R MASS package (Venables and Ripley 2002). The volume under these surfaces is normalized to 1 to facilitate unbiased comparisons between morphs.

Statistical Analyses of the Effects of Plant Genotype
We first used MANOVA to determine the effect of plant genotype, gall morphotype, and their interaction on gall thickness and diameter because we expected these traits to be somewhat correlated. A highly significant MANOVA allowed us to proceed with univariate ANOVAs for each trait separately. All analyses were conducted with basic core functions in R (R Development Core Team 2015) unless otherwise indicated, and R scripts are provided in the supplementary material, available online.

Table 1: Sample sizes (and number of parasitized galls) by attacking parasitoid and gall morphotype

| Parasitoid species     | Irregular | Crescent | Flat   | Cushion |
|------------------------|-----------|----------|--------|---------|
| Aprostocetus sp.       | 1,181 (87)| 814 (46) | 640 (72)| 508 (42)|
| Aprostocetus tesserus   | 1,181 (135)| 814 (99) | 640 (63)| 510 (49)|
| Aprostocetus homeri     | 1,181 (9) | 815 (11) | 640 (4) | 508 (4) |
| Baryscapus fumipennis  | 1,181 (20)| 814 (36) | 640 (29)| 508 (17)|
| Closterocerus solidaginis| 1,181 (142)| 814 (77) | 641 (212)| 508 (50)|
| Torymus capite         | 1,181 (26)| 814 (6) | 640 (85)| 508 (76)|
| Platygaster solidaginis| 1,181 (17)| 814 (104)| 640 (24)| 508 (21)|
| Total                  | 1,795 (1,030)| 1,228 (779)| 947 (732)| 784 (516)|

Statistical Analyses of Parasitism
Attack by each of the parasitoids was analyzed with a χ² test for independence. Pearson standardized residuals were plotted by gall morph and parasitoid, \((n_i - \mu_i)/\sqrt{\mu_i}\), where \(n\) and \(\mu\) are the observed and expected cell frequencies, respectively. Sample sizes and number parasitized for each of the gall morphotypes by parasitoid species are presented in table 1 (note that the total sample sizes and number parasitized are not the column sums because sometimes the parasitoid had already emerged at the time of gall collection, indicating parasitism by an unknown parasitoid). Empty galls could easily be recognized as parasitized by the circular shape of the parasitoid emergence hole. In contrast to parasitoids, Asteromyia adults leave an irregularly shaped emergence hole in the gall. Occasionally, we found instances where an identifiable parasitoid was present but with additional parasitoid emergence holes from other chambers, indicating that additional parasitoids were once in the gall. In these cases, it was not clear which parasitoid species emerged, and therefore the occurrence of the six other parasitoids could not be determined, resulting in slightly different sample sizes depending on the parasitoid species (table 1).

Visualizing the Shape of Selection
We used a generalized additive model (GAM, R package mgcv, gam function, link = logit) with Wood’s (2006) tensor product smooth to generate individual fitness functions. Default settings (i.e., \(k = 5\), bs = “tp”) were employed, and the gam.check function (R package mgcv) indicated that this was appropriate for all models. Population-level fitness landscapes were created by passing these GAM models to the fitness.landscape function in the R gsg package devel-
opened by Morrissey and Sakrejda (2013). The gsg package does not accept models with more than two traits; therefore, where number of larval chambers interacted significantly with gall thickness or diameter in the parametric models, individual GAM models were created for galls with only one, two, or three or more chambers. Cross sections at mean phenotype were also created with the fitness.landscape function in gsg using the points argument and 1,000 case bootstrap replicates to estimate prediction intervals (i.e., 25% and 75% quantiles of the bootstrap replicates). A similar visualization (but of individual fitness surfaces) was proposed and described mathematically by Schluter (1988) and has been used subsequently by others (e.g., Egan et al. 2011). It is a flexible nonparametric technique that makes no a priori assumptions about the shape of the individual fitness surface. In addition to examining each gall morph separately, we estimated fitness surfaces for all morphs pooled together to provide insight into the selective forces that may have been responsible for generating the observed diversity of morphotypes.

**Selection Gradient Analyses**

We used logistic regression and the methods of Janzen and Stern (1998) to calculate linear and quadratic selection gradients. These methods are similar to the traditional methods of Lande and Arnold (1983) but differ in their assumptions and treatment of binomial response variables, such as ours. Logistic regression is less sensitive to outliers and a more natural way to model binomial responses. Parametric methods can lead to incorrect inferences, especially with respect to quadratic selection gradients (see Schluter 1988). This is why we verified the curvature detected in our parametric models with GAMs, which also suggested the inclusion of cubic terms in the parametric model.

The parametric logistic regression model used was

$$\logit(E(W)) = \mu + \beta_d + \beta_t + \beta_c + \gamma_{d^2} + \gamma_{t^2} + \gamma_{c^2} + \delta_{dc} + \delta_{dt} + \delta_{tc} + \gamma_{da} + \gamma_{ta} + \gamma_{ca} + \delta_{dta},$$

where $W$ denotes absolute fitness and $d$, $t$, and $c$ denote standardized gall diameter, thickness, and number of larval chambers, respectively (R glm function, link = logit). This full model was reduced to a simpler model with the stepAIC function (R package, MASS), and the directional selection gradients were estimated with a model with only linear terms. In no cases were terms that were significant in the full model eliminated by stepAIC. All coefficients (and standard errors) were divided by mean survival and adjusted according to Janzen and Stern (1998) to obtain standardized selection gradients based on relative fitness. The quadratic coefficients and their corresponding standard errors were doubled to obtain proper quadratic selection gradients (see Lande and Arnold 1983; Janzen and Stern 1998; Stinchcombe et al. 2008; Haller and Hendry 2014). Cubic terms were adjusted as above but not doubled.

**Calculating the Fitness Response and Other Methodological Considerations**

Individual gall survival was calculated by dividing the number of healthy midges found in the gall by the number of larval chambers to average the effects of clutch size, avoid pseudoreplication, and estimate survival on a per larva basis rather than a per gall basis. Crescents always lay only one egg, and thus survival on a per gall and a per larval basis is a binomial response of presence or absence of parasitism where absence was coded as 1. The other gall morphs form galls with varying numbers of eggs (i.e., chambers), and thus survival can be some proportion of 1. In both logistic regression and the GAM models, the logit link argument was specified because all survival measurements were bounded between 0 and 1. Occasionally, galls fail to produce a chamber and the midge larva(e) dies early during development. These represented <12% of gall records and were excluded from the analysis.

**Potential Cryptic Effects on Gall Morphology**

Parasitism itself may affect gall morphology and potentially confound phenotypic selection on midge lineages. Direct observations of oviposition have confirmed that females often lay more than one clutch (i.e., gall) on an individual leaf. We tested whether such effects are likely to bias our analyses by finding instances in our data set where parasitized and unparasitized galls occurred on the same leaf of the same morph and at the same time. Using this reduced data set, we compared whether these sibling galls differed in morphology when parasitized with paired $t$-tests.

**Results**

*How Do Gall Morphologies Differ Phenotypically?*

Kernel density distributions indicated that gall morphology with respect to gall diameter and thickness is roughly multivariate normal regardless of the gall morph considered. Crescents and irregulars are closest to the overall mean phenotype (fig. 1), whereas flats and cushions depart from the mean substantially, with cushions being the thickest and flats having greater diameter (Grego et al. 1990; Stireman et al. 2008). The greatest morphological distance is between irregulars and cushions. ANOVAs indicated that there were
significant differences between gall morphotypes in diameter and thickness (fig. 2).

Overall, *Asteromyia carbonifera* galls experienced heavy mortality (>60%) from parasitoids; however, there was considerable variation in parasitism rates between gall morphs. Logistic regression analysis of overall survival indicated that irregulars and cushions (the two most divergent morphs) experienced significantly lower parasitism than crescents and flats, and crescents experienced lower parasitism than flats (fig. 1, inset; df = 3, 4,739, deviance = 68.6, $P \ll .001$).

What Is the Effect of Plant Genotype on Gall Morphology?

Gall thickness and gall diameter were both significantly affected by plant genotype, but the effect size was small relative to the effect of gall morph (fig. 2). At most, the *Solidago altissima* genotype explained 6.8% of the variation in gall thickness and only 3.6% of the variation in gall diameter, including the interaction term. However, gall morph explained 49.1% of variation in gall thickness and 33.6% of variation in gall diameter (for ANOVA results, see fig. 2), illustrating that most of the variation in gall morphology is due to midge genotype.

Do Parasitoids Use Alternative Gall Morphs?

We found strong, significant differences in parasitoid use of different gall morphs (fig. 3; table 1). Each of the parasitoid taxa except *Aprostocetus tesserus* and *Aprostocetus homeri* (a rare species) displayed significant differences in use of one or more gall morphs (*Aprostocetus* sp.: $\chi^2 = 16.2$, df = 3, $P = .001$; *A. tesserus*: $\chi^2 = 3.2$, df = 3, $P = .36$; *A. homeri*: $\chi^2 = 2.7$, df = 3, $P = .43$; *Baryscapus fumipennis*: $\chi^2 = 16.0$, df = 3, $P = .001$; *Closterocerus solidaginis*: $\chi^2 = 199$, df = 3, $P \ll .001$; *Torymus capite*: $\chi^2 = 198$, df = 3, $P \ll .001$; *Platygaster solidaginis*: $\chi^2 = 131$, df = 3, $P \ll .001$). Crescents were more heavily attacked by the egg parasitoid *P. solidaginis*, the larger and thicker gall morphs were used more by *T. capite*, and flats experienced much greater attack by *C. solidaginis* and the undescribed *Aprostocetus* sp. Irregulars were relatively protected from parasitoid attack.

What Is the Shape and Strength of Selection on Gall Morphology?

We found significant evidence for directional and disruptive selection on *A. carbonifera* gall morphology, but the
shape and strength of selection varied substantially among the genetically distinct gall morphotypes (figs. 4–6; table 2). Figures 4–6 present four key components in a common format across all figures: (1) fitness landscapes (i.e., three-dimensional surfaces), (2) cross sections through these same landscapes with corresponding prediction intervals, (3) the distribution of the trait before selection, and (4) selection differentials. The cross sections (i.e., two marginal figures per surface) give a profile of the adjacent surface at mean (i.e., zero) phenotype of the opposite trait as well as the distribution of the trait before selection (i.e., second solid line). In these marginal figures the solid line juxtaposed by dotted lines (i.e., first and third quartiles of 1,000 bootstrap replicates) gives an indication of the robustness of the survival probability, indicated by the dashed lines in figure 5D, as well as small $P$ values associated with disruptive and correlational selection in cushions on thickness (table 2). For crescent morphs, selection was for thinner galls ($\beta_t = -0.206$; table 2; fig. 4A), while for gall diameter, there was evidence of disruptive and cubic selection ($\gamma_d = 0.130$, $\delta_d = -0.021$; fig. 4B, 4C; table 2). In irregular morphs we found evidence of directional selection for a greater number of chambers ($\beta_c = 0.111$; table 2). We did not detect significant selection on flat morphs, but there were trends suggesting directional and disruptive selection on the number of chambers and gall diameter (table 2).

For the pooled analysis, including all morphs, strong selection was evident, especially disruptive (or divergent) selection on diameter (favoring small and large galls; $\gamma_d = 0.151$; table 2; fig. 6B, 6E, 6H) and thickness (favoring thin and thick galls; $\gamma_t = 0.198$; table 2; fig. 6A, 6D, 6G) and correlational selection on thickness and number of chambers (favoring thick galls but only in galls with few chambers; $\gamma_{tc} = -0.116$; table 2; fig. 6A, 6D, 6G). There was also weak but significant correlational selection on diameter and number of chambers (favoring small galls with few chambers or large galls with many chambers; $\gamma_{tc} = -0.055$; table 2) and the complicated three-way cubic interaction of all three traits ($\delta_{tbc} = 0.034$; table 2; fig. 6C, 6F, 6I). Comparing the fitness landscape surfaces in figures 4 and 5 with those in figure 6, it is evident that disruptive selection in the pooled analysis (i.e., fig. 6) emerges from a combination of disruptive selection on cushions plus the more complicated fitness surface in crescents. For instance, the surfaces in figures 5C and 6C are nearly identical, ex-
cept for the hump in the fitness landscape in figure 6 caused by the incorporation of crescents, whose galls never have more than one chamber. On the basis of the selection differentials and fitness landscapes, crescents are predicted to climb the fitness peak seen in figure 4C. The other graphs in figures 5 and 6 are constructed similarly, and using this same logic, cushions with a single chamber or three or more chambers are expected to get thicker. Furthermore, the thickness distribution of cushions with two chambers is expected to broaden, given the strong disruptive selection (fig. 5D, 5F).

Cryptic Effects on Gall Morphology

We identified hundreds of instances in our data set where galls of the same morph occurred on the same leaf at the same time. Using paired t-tests, we tested whether parasitism itself may have developmental effects on gall morphology and compared the effects with those of the overall selection gradients reported in table 2. Except for cushions, parasitism significantly affected the morphology of gall morphs (table 3) but in a direction that, if anything, biases our data set against the detection of selection (i.e., potential for type II errors). Thickness was generally increased when galls were parasitized, but not for cushions. Diameter was also generally increased when galls were parasitized, but again cushions and also crescents were unaffected.

Discussion

Predation can act as a source of divergent selection on antipredator traits and contribute to adaptive divergence between populations (Dieckmann et al. 2004; Nosil 2012). We measured selection on antipredator fungal phenotypes in a fly-fungus symbiosis experiencing a recent and ongoing radiation on Solidago altissima (Stireman et al. 2012) and demonstrate both directional and disruptive selection on gall phenotypes. Host plant variation at this level explains little variation in gall morphology; however, gall morphs exhibit strong heterogeneity in predator attack. Although we do not yet know whether and how this variation may contribute to reproductive isolation, Asteromyia carbonifera...
lineages appear to be diverging in (extended) phenotype due to selection imposed by enemies.

**Natural Enemies and Diverging Fungal Morphs**

Weis (1982a) proposed that the ecological significance of the morphological variation in fungal galls is related to differences in attack behavior and ovipositor length in parasitoid wasps that attacked them. Acting as the lethal analog of a pollinator’s tongue, parasitoid ovipositors are closely matched to the toughness and size of their victims. Mortality from parasitoid wasps might selectively favor extreme gall phenotypes that escape predation either because of apostatic selection (Bond 2007) or because parasitoids are unable to successfully parasitize divergent phenotypes (Weis 1982a). This idea is consistent with observations from other systems, such as siricid woodwasps (Heatwole and Davis 1965) and the fungal mutualist of some scale insects, *Septobasidium* (Couch 1931). Our results indicate strong differential use by five parasitoids of the divergent morphotypes,

### Table 2: Selection gradients for gall diameter, thickness, and number of chambers in multilocular galls

| Morph and selection gradient | Estimate | SE  | z    | P    |
|-----------------------------|----------|-----|------|------|
| Irregular (*n* = 1,770):    |          |     |      |      |
| β̂_d | −0.19 | 0.043 | −4  | .661 |
| β̂_t | 0.026 | 0.038 | 7.  | .494 |
| β̂_c | 0.111 | 0.041 | 2.7 | .007** |
| Crescent (*n* = 1,207):    |          |     |      |      |
| β̂_d | 0.117 | 0.053 | 2.2 | .028* |
| β̂_t | −0.206 | 0.055 | −3.7 | <.001*** |
| β̂_c | −0.008 | 0.057 | −1  | .886 |
| γ̂_d | 0.130 | 0.057 | 2.3 | .023* |
| δ̂_t | −0.021 | 0.010 | −2.0 | .043* |
| Flat (*n* = 935):           |          |     |      |      |
| β̂_d | −0.076 | 0.089 | −9  | .395 |
| β̂_t | −0.103 | 0.081 | −1.3 | .203 |
| β̂_c | 0.138 | 0.081 | 1.7 | .088* |
| γ̂_t | −0.217 | 0.126 | −1.7 | .083* |
| γ̂_tc | 0.155 | 0.086 | 1.8 | .072* |
| Cushion (*n* = 773):        |          |     |      |      |
| β̂_d | 0.146 | 0.071 | 2.1 | .040* |
| β̂_t | 0.228 | 0.060 | 3.8 | <.001*** |
| β̂_c | −0.052 | 0.069 | −8  | .450 |
| γ̂_t | 0.300 | 0.081 | 3.7 | <.001*** |
| γ̂_tc | 0.087 | 0.051 | 1.7 | .089* |
| γ̂_dc | −0.129 | 0.057 | −2.3 | .023* |
| δ̂_t | −0.014 | 0.009 | −1.6 | .113 |
| Pooled (*n* = 4,722):       |          |     |      |      |
| β̂_d | −0.093 | 0.033 | −2.8 | .004** |
| β̂_t | 0.050 | 0.029 | 1.7 | .084* |
| β̂_c | 0.063 | 0.029 | 2.2 | .028* |
| γ̂_t | 0.151 | 0.060 | 2.5 | .012* |
| γ̂_tc | 0.198 | 0.036 | 5.5 | <.001*** |
| δ̂_t | −0.011 | 0.007 | −1.6 | .116 |
| γ̂_dc | −0.003 | 0.036 | −1  | .943 |
| γ̂_dc | −0.055 | 0.028 | −2.0 | .051* |
| γ̂_tc | −0.116 | 0.038 | −3.0 | .003*** |
| δ̂_dc | 0.034 | 0.018 | 1.9 | .055* |

Note: Gradients were obtained from coefficients of multiple logistic regression of individual fitness on gall morphotype and for the morphotypes pooled. Selection gradients are standardized and indicated by directional (β), quadratic (e.g., γ), correlational (e.g., γ), and cubic (δ) selection on gall diameter (d), thickness (t), and number of larval chambers (c) in multilocular galls. Positive and negative estimates of quadratic gradients are suggestive of disruptive and stabilizing selection, respectively. Directional gradients were determined with logistic regression models with only linear terms.

* P ≤ .10.
** P ≤ .05.
*** P ≤ .001.

Adaptive Divergence Driven from the Top Down
with two additional species \((Aprostocetus homeri)\) and \((Aprostocetus tesserus)\) showing no significant differential use of morphs. The former is very rare, and so this may be an issue of statistical power, but \(A. tesserus\) is common. Perhaps \(A. tesserus\) (which attacks late during gall development and drills into the gall from the top) is unimpeded by any of the gall morphs on \(S. altissima\) (Weis 1982a).

Overall, significant selection gradients indicate that in \(A. carbonifera\), there is a fitness cost to producing certain gall shapes. Three of the gall morphs (irregular, crescent, and

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**Figure 5:** Population mean fitness of \(Asteromyia carbonifera\) cushion morphs as a function of gall diameter, thickness, and number of chambers. This figure includes an additional trait (number of chambers) but is otherwise similar to figure 4 (see fig. 4).
cushion) display strong evidence of current selection by parasitoids on the gall traits measured, and perhaps even more revealing, pooled analyses of all gall morphs reveal strong net disruptive selection on both thickness and diameter. More detailed analyses of how the mode and strength of selection exerted on gall traits varies among parasitoid taxa are ongoing. However, it is clear from our initial analyses that parasitoid species contribute differently to the net selection surfaces reported here (J. J. Heath, P. Abbot, and J. O. Stireman III, unpublished data). Traits such as diameter, color, and reflectance are likely to influence the ability of parasitoids to locate and recognize galls, and traits such as thickness and hardness are

Figure 6: Population mean fitness of Asteromyia carbonifera pooled morphs as a function of gall diameter, thickness, and number of chambers. This figure is similar to figure 5 but includes all morphs pooled (see figs. 4, 5).
likely to directly influence handling time and oviposition success (Abrahamson and Weis 1997). Parasitoids may select for a greater number of chambers if gall size and thickness limit access to larvae or if wasps are likely to leave galls after a single successful parasitism event (e.g., when gall densities are high and most galls are unilocular), or they may select for fewer chambers if multilocular galls are more apparent, gall densities are low, and galls frequently harbor many host larvae (Godfray 1994). If multilocular galls increase parasitism risk per host, they may benefit relatively more from defensive traits, such as gall thickness. Although more work is needed to unveil the behavioral, sensory, and physical mechanisms driving these selection gradients, this contemporary selection implies that gall morph phenotype has been historically shaped by parasitoid pressure and may have resulted in the observed pattern of variation in gall morphology.

Gall morphology, however, is not solely described by diameter, thickness, and number of chambers. Other morphological and even behavioral traits are likely to be important as well (Stone and Schonrogge 2003). Among the four gall morphs studied here, irregulars are the most divergent in form (as the name suggests) and, interestingly, suffer the lowest parasitism levels. Irregulars usually have two larvae per gall, but their larval chambers are characteristicly positioned at the edge of the gall rather than in the center, as in other morphs. Irregulars also have morphological features not readily encompassed by diameter and thickness. For instance, the thick, hard layer of black fungal stroma that underlays the outer surface of all gall morphs usually does not penetrate the leaf mesophyll. In irregulars, this stroma penetrates the mesophyll and may provide a protective barrier against parasitoids that insert their ovipositors from the edge of the gall, such as Torymus capite (Weis 1982a, 1982b, 1983). Crescent morphs are also distinct in laying their eggs on the edge of leaves and on both young and mature tissue, the latter of which provides significant protection from the egg parasitoid, Platygaster solidaginis (Heath et al. 2013).

### Table 3: Results of paired t-tests of morphological trait differences between mature parasitized and unparasitized Asteromyia carbonifera galls occurring on the same Solidago altissima leaf at the same time

| Morph  | Difference (mm) | CI (95%) | Difference (SD) | df | t    | P    | Potential for bias |
|--------|-----------------|---------|----------------|----|------|------|-------------------|
| Thickness: |                |         |                |    |      |      |                   |
| Irregular | .020          | .002–.037 | .155         | 203 | 2.25 | .025 | Type Ic           |
| Crescent  | .056          | .014–.098 | .412         | 54  | 2.68 | .010** | Type Ib          |
| Flat      | .101          | .046–.157 | .492         | 37  | 3.68 | .001*** | Type Ib       |
| Cushion   | −.005         | −.066 to .057 | −.014   | 90  | −.15 | .880 | NA              |
| Diameter: |                |         |                |    |      |      |                   |
| Irregular | .187          | .053–.320 | .188         | 205 | 2.75 | .010** | Type Ib          |
| Crescent  | .115          | −.112 to .343 | .123   | 54  | 1.02 | .315 | NA              |
| Flat      | 1.255         | .605–1.905 | .873        | 38  | 3.91 | .001*** | Type Ib    |
| Cushion   | .129          | −.291 to .549 | .072   | 92  | .61  | .544 | NA              |

Note: Differences are between parasitized and unparasitized galls. The trend is for galls to be thicker when parasitized. Potential bias on selection gradients. CI, confidence interval; NA, not applicable (not significantly different and effect sizes small).

* The only significant irregular gradient is directional, selecting for a greater number of chambers. More chambers would increase the diameter, which is the opposite of the effect here (i.e., only potential for type II error).

** The effect of parasitism may cause an overestimation of the negative directional selection gradient on thickness for crescents (i.e., potential for type I error).

*** None of the selection gradients are significant for flats, therefore, only the potential for type II errors.

P ≤ .05.

P ≤ .01.

P ≤ .001.

Indirect Effects of Host Plants and Other Cryptic Causes of Variation in Gall Morphology

Plant genotype may have indirect effects on parasitism rates through its influence on gall thickness and diameter but is weak with respect to the effect of midge larvae on gall morphology. Nevertheless, even small differences in gall thickness and diameter could conceivably encourage host plant specialization if they reduce vulnerability of midges to parasitoids. Because only successful galls were included in our analyses, the effects of host plant resistance on gall survival were removed. Parasitism itself could affect gall morphology because it may disrupt midge-fungal interactions. However, we failed to find evidence that this would significantly bias our results. In all but one case, either parasitism would render our selection estimates more conservative or it was not statistically significant. Thicker crescent morphs were more likely to be parasitized (table 3), perhaps explaining evidence of selection for thinner galls in this morph, but this pattern may also be due to parasitoids preferring to oviposit in thicker galls that are more likely to
contain large, healthy larvae. Our findings that parasitoids select for thicker galls in some cases and thinner galls in others as well as the strong effects of number of chambers on fitness surfaces indicate that little if any of the observed variation in gall morphology is directly due to the parasitism status of the gall. In fact, evidence of correlational selection on chamber number and gall shape (e.g., cushions) suggests genetic covariation between traits; however, we were unable to evaluate this possibility because of experimental difficulties this system poses.

Top-Down Effects and Speciation

Most ecological models of speciation invoke diversifying selection as a critical first step in generating the phenotypic diversity that fuels the speciation process, but a major challenge has been to detect evidence of diversifying selection in natural populations that may be driving speciation (Bolnick 2004). Top-down perspectives can generate novel hypotheses concerning adaptive diversification (Singer and Stireman 2005). However, teasing apart the relative roles of predators and other factors (e.g., host plants or geographical isolation) in diversification is a complex problem. Even under intense diversification selection, barriers to gene flow among populations are necessary for adaptive radiation to proceed (Coyne and Orr 2004; although see Nosil 2008; Nosil et al. 2009). For example, the classic adaptive radiation of Geospiza finches in the Galapagos Islands, while associated with selection on beak shape due to variation in resources (Grant and Grant 1993, 2006), is at least partially dependent on the isolation of populations on different islands (Petren et al. 2005). Analogously, parasitoids may select for diversifying gall phenotypes in A. carbonifera, but by itself this pressure is unlikely to result in genetic divergence among populations. Additional isolating factors fostering population differentiation may be related to habitat patchiness, Solidago host plant populations, species, or their interaction.

In the genus Asteromyia, host plant–associated diversification has resulted in much cryptic diversity and has been important in the adaptive divergence of the clade (Stireman et al. 2010, 2012). Initial population divergence of A. carbonifera morphotypes on S. altissima may have occurred allopatrically in semi-isolated habitat patches in response to local parasitoid communities. Once sympatry was reestablished, additional morphological divergence could be driven by apparent competition via shared parasitoids (Holt and Lawton 1993). Another possibility is that initial phenotypic divergence of midges may have occurred after a shift to a new host plant species (Stireman et al. 2012). Novel host plants may provide at least partial reproductive isolation and create novel selective environments associated with distinct enemy communities, which could lead to novel gall phenotypes. If these phenotypes are sufficiently divergent from the ancestor, apparent competition between the two populations via shared parasitoids may be reduced to the extent that shifts back to the ancestral host plant are favored, resulting in the co-occurrence of distinct morphs on a single plant species. Further work is needed to evaluate these possibilities and identify the barriers to gene flow among morphs.

Diversifying selection from the top down, as shown here, is likely an underappreciated cause of adaptive diversification in gall-forming insect lineages generally. Like the fungal galls of Asteromyia midges, plant galls of insect herbivores are extended phenotypes of alternative gall-maker genotypes (Crespi and Worobey 1998). A primary function of variation in gall morphology is defense (Stone and Schonrogge 2003), and a number of lineages have diversified extensively within a single host plant species (Jones et al. 1983; Cook et al. 2002; Joy and Crespi 2007). Even more broadly, selection by enemies may play important roles in diversification of phytophagous insects as a whole (Singer and Stireman 2005), given that their populations are typically limited less by resources and more by enemies (Hairston et al. 1960).

Conclusions

A growing body of theory exists on how predators can shape the outcome of competition and mate choice in phenotypically variable or habitat segregating populations, thereby influencing character shifts, co-existence, and/or divergence (Holt 1977; Jeffries and Lawton 1984; Abrams 2000; Doebeli and Dieckmann 2000). Authors have noted the need for empirical inquiry into the role of consumer-resource interactions in evolutionary divergence, motivated by the obvious effects that predators and parasites have on the biology of their prey (Buckling and Rainey 2002; Rundle et al. 2003; Vamosi and Schluter 2004; Singer and Stireman 2005; Vamosi 2005; Langerhans et al. 2007; Meyer and Kassen 2007; Karvonen and Seehausen 2012). Even in herbivorous insects, where natural selection imposed from the bottom up (plants) and the top down (natural enemies) has long been formalized under a tritrophic niche concept, the central questions have concerned the relative roles of plants and predators in herbivore population dynamics and coexistence (Hairston et al. 1960; Price et al. 1980). Whether natural enemies frequently act as initiating, primary agents that foster adaptive radiation (rather than as secondary modifiers of trait values expressed along axes of resources or sex) remains a question in need of additional empirical study (Nosil and Crespi 2006; Craig et al. 2007a, 2007b; Bailey et al. 2009). Our results suggest that natural enemies can foster divergence in phenotypes underlying adaptive ecological and genetic divergence, likely fueling the explosive biological diversification of their prey (Stireman et al. 2008, 2010, 2012).
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Literature Cited
Abrahamson, W. G., and A. E. Weis. 1997. Evolutionary ecology across three trophic levels: goldenrods, gallmakers, and natural enemies. Princeton University Press, Princeton, NJ.
Abrams, P. 2000. Character shifts of prey species that share predators. American Naturalist 156(suppl.):S45–S61.
Bailey, R., K. Schorrogge, J. M. Cook, G. Melika, G. Csoka, C. Thuroczy, and G. N. Stone. 2009. Host niches and defensive extendedphenotypes structure parasitoid wasp communities. PLoS Biology 7:e1000179.
Bissett, J., and A. Borkent. 1988. Ambrosia galls: the significance of fungal nutrition in the evolution of the Cecidomyiidae. Pages 203–225 in K. A. Pirozynski and D. L. Hawksworth, eds. Coevolution of fungi with plants and animals. Academic Press, San Diego, CA.
Bolnick, D. 2004. Can intraspecific competition drive disruptive selection? an experimental test in natural populations of sticklebacks. Evolution 58:608–618.
Bond, A. B. 2007. The evolution of color polymorphism: crypticity searching images, and apostatic selection. Annual Review of Ecology, Evolution, and Systematics 38:489–514.
Borkent, A., and J. Bissett. 1985. Gall midges (Diptera: Cecidomyiidae) are vectors of their fungal symbionts. Symbiosis 1:185–194.
Brucker, R. M., and S. R. Bordenstein. 2012. Speciation by symbiosis. Trends in Ecology and Evolution 27:443–451.
Buckling, A., and P. B. Rainey. 2002. The role of parasites in sympatric and allopatric host diversification. Nature 420:496–499.
Cain, A., and P. Sheppard. 1954. Natural selection in Cepaea. Genetics 39:89–116.
Clarke, B. 1969. Evidence for apostatic selection. Heredity 24:347–352.
Cook, J. M., A. Rokas, M. Pagel, and G. N. Stone. 2002. Evolutionary shifts between host oak sections and host-plant organs in Andricus galls wasps. Evolution 59:1821–1830.
Couch, J. N. 1931. The biological relationship between Septobasidium retiforme (B. & C.) Pat. and Aspidiatus obsorni New. and Ckll. Quarterly Journal of Microscopical Science 74:383–438.
Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer, Sunderland, MA.
Craig, T. P., J. K. Itami, and J. V. Craig. 2007a. Host plant genotype influences survival of hybrids between Eurosta solidaginis host races. Evolution 61:2607–2613.
Craig, T. P., J. K. Itami, and J. D. Horner. 2007b. Geographic variation in the evolution and coevolution of a tritrophic interaction. Evolution 61:1137–1152.
Crego, C. L., A. E. Weis, N. O. Polans, and C. K. Bretz. 1990. Sympatric sibling species from three phenotypically distinct Asteromyia (Diptera, Cecidomyiidae) galls on the same host plant species. Annals of the Entomological Society of America 83:149–154.
Crespi, B., and M. W. Worobey. 1998. Comparative analysis of gall morphology in Australian gall thrips: the evolution of extended phenotypes. Evolution 52:1686–1696.
Dieckmann, U., M. Doebeli, J. A. J. Metz, and D. Tautz, eds. 2004. Adaptive speciation. Cambridge University Press, Cambridge.
Doebeli, M., and U. Dieckmann. 2000. Evolutionary branching and sympatric speciation caused by different types of ecological interactions. American Naturalist 156(suppl.):S77–S101.
Egan, S. P., G. R. Hood, and J. R. Ott. 2011. Natural selection on gall size: variable contributions of individual host plants to population-wide patterns. Evolution 65:3543–3557.
Fish, R. A. 1958. The genetical theory of natural selection. Dover, New York.
Godfray, H. C. J. 1994. Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton, NJ.
Grant, B. R., and P. R. Grant. 1993. Evolution of Darwin’s finches caused by a rare climatic event. Proceedings of the Royal Society B 251:111–117.
Grant, P. R., and B. R. Grant. 2006. Evolution of character displacement in Darwin’s finches. Science 313:224–226.
Hairson, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control, and competition. American Naturalist 94:421–425.
Haller, B. C., and A. P. Hendry. 2014. Solving the paradox of stasis: squashed stabilizing selection and the limits of detection. Evolution 68:483–500.
Heath, J. J., P. Abbot, and J. O. Stireman III. 2018. Data from: Adaptive divergence in a defense symbiosis driven from the top down. American Naturalist, Dryad Digital Repository, http://dx.doi.org/10.5061/dryad.p49b1.
Heath, J. J., A. Kessler, E. Woebbe, D. F. Cipollini, and J. O. Stireman III. 2014. Exploring plant defense theory in tall goldenrod, Solidago altissima. New Phytologist 202:1357–1370.
Heath, J. J., and J. O. Stireman III. 2010. Dissecting the association between a gall midge, Asteromyia carbonifera, and its symbiotic fungus, Botryosphaeria dothidea. Entomologia Experimentalis et Applicata 137:36–49.
Heath, J. J., B. Wells, D. Cipollini, and J. O. Stireman III. 2013. Carnivores and carotenoids are associated with adaptive behavioural divergence in a radiation of gall midges. Ecological Entomology 38:11–22.
Heatwole, H., and D. M. Davis. 1965. Ecology of three sympatric species of parasitic insects of the genus Megarhyssa (Hymenoptera, Ichneumonidae). Ecology 46:140–150.
Holt, R. D. 1977. Predation, apparent competition, and structure of prey communities. Theoretical Population Biology 12:197–229.
Holt, R. D., and J. H. Lawton. 1993. Apparent competition and enemy-free space in insect host-parasitoid communities. American Naturalist 142:623–645.
Janson, E. M., R. J. Grebenok, S. T. Behmer, and P. Abbot. 2009. Same host-plant, different sterols: variation in sterol metabolism in an insect herbivore community. Journal of Chemical Ecology 35:1309–1319.
Jansen, E. M., E. R. Peeden, J. O. Stireman III, and P. Abbot. 2010. Symbiont-mediated phenotypic variation without co-evolution in an insect-fungus association. Journal of Evolutionary Biology 23:2212–2228.

Janzen, D. H. 1967. Population dynamics in tropical rain forests. Science 158:258–266.

Kettlewell, B. 1973. The evolution of melanism: the study of a recurring phenomenon. Clarendon, Oxford.

Kingsolver, J., H. Hoekstra, J. Hoekstra, D. Berrigan, S. Vignieri, C. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. American Naturalist 157:245–261.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Langerhans, R. B. 2006. Evolutionary consequences of predation: avoidance, escape, reproduction, and diversification. Pages 177–220 in A. M. T. Elewa, ed. Predation in organisms: a distinct phe-

Langerhans, R. B., M. E. Gifford, and E. O. Joseph. 2007. Ecological speciation in Gambusia fishes. Evolution 61:2056–2074.

Marchinko, K. B. 2009. Predation and phenotypic variation in an insect-fungus association. Journal of Evolutionary Biology 23:2212–2228.

Jeffries, M. J., and J. H. Lawton. 1984. Enemy free space and the structure of ecological communities. Biological Journal of the Linnean Society 23:269–286.

Jones, R. G., R. J. Gagné, and W. F. Barr. 1983. Biology and taxonomy of the Rhopalomyia gall midges (Diptera: Cecidomyiidae) of Arctesia tridentata Nutall (Compositae) in Idaho. Contributions of the American Entomological Institute 21:1–76.

Kettlewell, B. 1973. The evolution of melanism: the study of a recurring necessity; with special reference to industrial melanism in the Lepidoptera. Clarendon, Oxford.

Kingsolver, J., H. Hoekstra, J. Hoekstra, D. Berrigan, S. Vignieri, C. Hill, A. Hoang, P. Gibert, and P. Beerli. 2001. The strength of phenotypic selection in natural populations. American Naturalist 157:245–261.

Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. Evolution 37:1210–1226.

Langerhans, R. B. 2006. Evolutionary consequences of predation: avoidance, escape, reproduction, and diversification. Pages 177–220 in A. M. T. Elewa, ed. Predation in organisms: a distinct phenomenon. Springer, Heidelberg.

Langerhans, R. B., M. E. Gifford, and E. O. Joseph. 2007. Ecological speciation in Gambusia fishes. Evolution 61:2056–2074.

Marchinko, K. B. 2009. Predation’s role in repeated phenotypic and genetic divergence of armor in threespine stickleback. Evolution 63:127–138.

Martin, R. A., and D. W. Pfennig. 2009. Disruptive selection in natural populations: the roles of ecological specialization and resource competition. American Naturalist 174:268–281.

Meyer, J. R., and R. Kassen. 2007. The effects of competition and predation on diversification in a model adaptive radiation. Nature 446:432–435.

Momen, G. 1962. Reflexive selection: a possible answer to an old puzzle. Science 136:262–263.

Morrissey, M. B., and K. Sakrejda. 2013. Unification of regression-based methods for the analysis of natural selection. Evolution 67:2094–2100.

Mullen, S. P., and K. L. Shaw. 2014. Insect speciation rules: unifying concepts in speciation research. Annual Review of Entomology 59:339–361.

Nosil, P. 2008. Speciation with gene flow could be common. Molecular Ecology 17:2103–2106.

Nosil, P., E. R. Peeden, and J. O. Stireman III. 2005. The tri-trophic niche concept and adaptive radiation of phytophagous insects. Ecology Letters 8:1247–1255.

Rueffler, C., T. Van Dooren, O. Leimar, and P. Abrams. 2006. Disruptive selection and then what? Trends in Ecology and Evolution 21:238–245.

Rundle, H. D., S. M. Vamosi, and D. Schluter. 2003. Experimental test of predation’s effect on divergent selection during character displacement in sticklebacks. Proceedings of the National Academy of Sciences of the USA 100:14943–14948.

Schluter, D. 1995. Adaptive Divergence Driven from the Top Down E35

Oliver, K. M., A. H. Smith, and J. A. Russell. 2014. Defensive symbiosis in the real world—advancing ecological studies of heritable, protective bacteria in aphids and beyond. Functional Ecology 28:341–355.

Parchman, T., and C. Benkman. 2002. Diversifying coevolution between crossbills and black spruce on Newfoundland. Evolution 56:1663–1672.

Paulson, D. 1973. Predator polymorphism and apostatic selection. Evolution 27:269–277.

Petren, K., P. Grant, B. Grant, and L. Keller. 2005. Comparative landscape genetics and the adaptive radiation of Darwin’s finches: the role of peripheral isolation. Molecular Ecology 14:2943–2957.

Price, P. W., and K. M. Clancy. 1986. Interactions among three trophic levels: gall size and parasitoid attack. Ecology 67:1593–1600.

Price, P. W., J. N. Thompson, A. E. Weis, B. A. McPherson, C. E. Boutron, and P. Gross. 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. Annual Review of Ecology and Systematics 11:41–65.

R Development Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

Ricklefs, R., and K. O’Rourke. 1975. Aspect diversity in moths: a temperate-tropical comparison. Evolution 29:313–324.

Rueffler, C., T. Van Dooren, O. Leimar, and P. Abrams. 2006. Disruptive selection and then what? Trends in Ecology and Evolution 21:238–245.

Rundle, H. D., S. M. Vamosi, and D. Schluter. 2003. Experimental test of predation’s effect on divergent selection during character displacement in sticklebacks. Proceedings of the National Academy of Sciences of the USA 100:14943–14948.

Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. Evolution 42:849–861.

———. 2000. The ecology of adaptive radiation. Oxford University Press, New York.

Singer, M. S., and J. O. Stireman III. 2005. The tri-trophic niche concept and adaptive radiation of phytophagous insects. Ecology Letters 8:1247–1255.

Stinchcombe, J. R., A. F. Agrawal, P. A. Hohenlohe, S. J. Arnold, and M. W. Blows. 2008. Estimating nonlinear selection gradients using quadratic regression coefficients: double or nothing? Evolution 62:2435–2440.

Stireman, J. O., III, H. Devlin, and P. Abbot. 2012. Rampant host-and defensive phenotype-associated diversification in a goldenrod gall midge. Journal of Evolutionary Biology 25:1991–2004.

Stireman, J. O., III, H. Devlin, T. G. Carr, and P. Abbot. 2010. Evolutionary diversification of the gall midge genus Asteromyia (Cecidomyiidae) in a multitrophic ecological context. Molecular Phylogenetics and Evolution 54:194–210.

Stireman, J. O., III, E. M. Janson, T. G. Carr, H. Devlin, and P. Abbot. 2008. Evolutionary radiation of Asteromyia carbonifera (Diptera: Cecidomyiidae) gall morphotypes on the goldenrod Solidago altissima (Asteraceae). Biological Journal of the Linnean Society 95:840–858.

Stone, G. N., and K. Schonrogge. 2003. The adaptive significance of insect gall morphology. Trends in Ecology and Evolution 18:512–522.

Svensson, E. I., and M. Friberg. 2007. Selective predation on wing shape in the flower beetle Apate metcalfi. Oikos 114:989–995.

Vamosi, S. M. 2005. On the role of enemies in divergence and diversification of prey: a review and synthesis. Canadian Journal of Zoology 83:894–910.
Vamosi, S. M., and D. Schluter. 2004. Character shifts in the defensive armor of sympatric sticklebacks. Evolution 58:376–385.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S. Springer, New York.

Weis, A. E. 1983. Patterns of parasitism by Torymus capite on hosts distributed in small patches. Journal of Animal Ecology 52:867–878.

———. 1982a. Resource utilization patterns in a community of gall attacking parasitoids. Environmental Entomology 11:809–815.

———. 1982b. Use of a symbiotic fungus by the gall maker Asteromyia carbonifera to inhibit attack by the parasitoid Torymus capite. Ecology 63:1602–1605.

Weis, A. E., and W. Abrahamson. 1986. Evolution of host-plant manipulation by gall makers—ecological and genetic factors in the Solidago-Eurosta system. American Naturalist 127:681–695.

Weis, A. E., P. W. Price, and M. Lynch. 1983. Selective pressures on clutch size in the gall maker Asteromyia carbonifera. Ecology 64:688–695.

Wood, S. N. 2006. Low-rank scale-invariant tensor product smooths for generalized additive mixed models. Biometrics 62:1025–1036.