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Indirect Interactions Shape Selection in a Multispecies Food Web

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ABSTRACT: Species do not live, interact, or evolve in isolation but are instead members of complex ecological communities. In ecological terms, complex multispecies interactions can be understood by considering indirect effects that are mediated by changes in traits and abundances of intermediate species. Interestingly, traits and abundances are also central to our understanding of phenotypic selection, suggesting that indirect effects may be extended to understand evolution in complex communities. Here we explore indirect ecological effects and their evolutionary corollary in a well-understood food web comprising a plant, its herbivores, and enemies that select for opposite defensive phenotypes in one of the herbivores. We show that ecological indirect interactions are mediated by changes to both the traits and the abundances of intermediate species and that these changes ultimately reduce enemy attack and weaken selection. We discuss the generality of the link between indirect effects and selection. We go on to argue that local adaptation and eco-evolutionary feedback may be less likely in complex multispecies food webs than in simpler food chains (e.g., coevolution). Overall, considering selection in complex interaction networks can facilitate the rapprochement of community ecology and evolution.

Keywords: Eurosta, goldenrod, trophic, plant-animal, eco-evo, diffuse coevolution.

Introduction

Species do not live in isolation but rather are members of complex and diverse communities. Despite this complexity, direct pairwise interactions among enemies, competitors, and mutualists form the building blocks of most ecological and evolutionary concepts. To understand complex communities and move beyond these building blocks, ecologists often invoke indirect effects: the modification of a pairwise interaction by the presence of a third species (Paine 1966; Strauss 1991). In fact, the importance of indirect effects may outweigh that of direct effects for determining species abundances and ultimately community composition (Menge 1995; Shurin et al. 2002). Although these ecological consequences of indirect effects are increasingly well understood, the evolutionary corollary has received comparatively little attention, hampering our ability to predict and understand selection in complex multispecies communities (Inouye and Stinchcombe 2001; terHorst et al. 2015, 2018; De Meester et al. 2016). Despite clear links between ecological and evolutionary indirect effects and the broader calls for the integration of community ecology and evolution (Antonovics 1992; Inouye and Stinchcombe 2001; Johnson and Stinchcombe 2007; Bolnick et al. 2011; Hendry 2016), surprisingly little work has aimed to integrate ideas about indirect effects in both fields.

Indirect ecological interactions are likely to affect evolutionary processes because both species interactions and selection depend on the traits and abundances of the focal organism and the other interacting organisms (McPeek 2017). Ecological indirect effects can occur when one species affects the abundance or traits of another, ultimately influencing the abundance or traits of a third species (Strauss 1991; Shurin et al. 2002). For example, spider predators reduce the abundance and behavior (traits) of grasshoppers (a herbivore), with the joint effects of these changes in abundance and traits reducing the impact of grasshoppers on plant resources (Schmitz et al. 1997; Genua et al. 2017). By shifting the abundance and behavior (traits) of grasshoppers, spider predators likely also alter patterns of selection imposed on plants by their herbivores. Our understanding of species interactions and hence selection from a perspective of traits and abundances (McPeek 2017) can facilitate an integration of ecological and evolutionary thinking in multispecies communities, particularly with respect to indirect interactions.

Diffuse coevolution is one process that links indirect interactions to evolution in multispecies communities. Diffuse coevolution is simply the idea that the presence of one species can alter the magnitude or direction of selection and ultimately coevolution between others (Janzen 1980; Inouye and Stinchcombe 2001). For example, resistance to flea beetle herbivory on wild mustard was favored when damage by a moth herbivore was low but was selected against when moth herbivory was high (Pilson 1996). In general, diffuse coevolution more often than not considers the evolutionary effects of multiple interacting species on one focal species ("interaction

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modification"; Inouye and Stinchcombe 2001; see above example), although other species arrangements are rarely considered (Abdala-Roberts et al. 2014). Conversely, ecological indirect effects more frequently consider the effect of species separated by intermediaries ("interaction chains"). A classic example is the trophic cascade, which can be traced through three or more trophic levels (species) with ultimate consequences for basal resources (Shurin et al. 2002). In short, diffuse coevolution and indirect effects tend to consider different arrangements of species interactions (although exceptions do exist; Inouye and Stinchcombe 2001), but ultimately both act by changing traits or abundances (McPeek 2017).

Trophic systems can form complex interaction webs, creating the potential for strong ecological and evolutionary indirect effects. Indirect interactions are prevalent in many food webs with a wide variety of interaction types and network topologies. To understand the details and nuances of selection acting on complex food webs, it will likely be necessary to study well-understood systems where indirect interactions are likely to be common. Owing to the specialization of many arthropod species and the herbivore-mediated plasticity of plant traits, plant-arthropod communities may be particularly prone to indirect effects (Ohgushi 2005, 2008). For example, a large literature has demonstrated that the presence of a herbivore can alter plant quality (defense or nutritional changes), indirectly affecting the abundance or traits of other arthropod herbivores (interaction modification; reviewed in Ohgushi 2005). Herbivores are themselves often attacked by suites of natural enemies, including parasitoids and predators. If enemy attack is density dependent (Holling 1959; Cappuccino 1992) and indirect effects between herbivores reduce the abundance of a focal herbivore (Hufbauer and Root 2002; Cunan et al. 2015), then attack by enemies should decline, reducing mortality and altering selection on herbivore defensive traits (density- or abundance-mediated effect in an interaction chain). However, enemies are rarely agnostic with respect to which herbivores they consume, preferring those with particular (poorly defended) phenotypes (e.g., Weis and Abrahamson 1985). If, rather than affecting abundance, one herbivore species affects the traits of another (Ohgushi 2008; Cunan et al. 2015), then this indirect effect may alter attack rates or the relationship between traits and fitness, ultimately shifting patterns of selection (trait-mediated effect). Complex indirect interactions may ripple through food webs by altering either traits or abundances of intermediate species, ultimately structuring selection.

In this study, we test for the ecological and evolutionary effects of indirect interactions using a well-studied plant, its herbivores, and their enemies. Goldenrod is attacked by herbivores, including leaf chewers such as the larvae of goldenrod leaf beetles (Cunan et al. 2015), and gall makers, including *Eurosta solidaginis* (hereafter *Eurosta*), which forms a spherical gall on goldenrod stems (fig. 1; Uhler 1951). Goldenrod leaf beetle herbivory has been shown to reduce plant root, stem, and leaf mass, reducing plant quality (Hufbauer and Root 2002), in turn reducing the abundance and/or size of *Eurosta* galls (fig. 1, prediction ii; Cunan et al. 2015). These changes in gall abundance and/or traits (size) may alter patterns of attack by natural enemies (fig. 1, predictions iii and iv), which include bird predators and hymenopteran parasitoids that preferentially attack large and small galls, respectively (interaction chain; Weis and Abrahamson 1985). Changes in the attack rate of these phenotype-selective enemies may then alter patterns of directional and nonlinear selection (fig. 1, prediction v; Weis and Abrahamson 1985; Start and Gilbert 2016). In sum, previous results from this well-studied system suggest that the effect of the leaf herbivore may alter the traits and abundances of the stem-galling herbivore and consequently shift patterns of attack, mortality, and selection.

We use an experimental approach to test the above ideas by hypothesizing that (i) high leaf beetle density will increase herbivory, (ii) this leaf herbivory will reduce the number and/or size of galls, (iii) lower gall abundance will reduce attack by all enemies, (iv) reduced gall size will increase attack by hymenopteran parasitoids but decrease attack by bird predators, and (v) these changes in attack rates will alter patterns of directional and nonlinear selection acting on gall size (all predictions are graphically summarized in fig. 1). Ultimately, we demonstrate the utility of indirect effects for understanding congruent ecological and evolutionary patterns in complex multispecies communities.

Methods

Study System

Goldenrod is a common old-field plant that is consumed by a large community of herbivores, a dominant member of which is the goldenrod leaf beetle (*Trirhabda canadensis*), which can defoliate in excess of 75% of the leaf surface area (Hufbauer and Root 2002; Cunan et al. 2015). Goldenrod is also attacked by *Eurosta*, a univoltine fly. Adult female *Eurosta* emerge from galls, mate, and oviposit in early June in southern Ontario, with galls appearing roughly 3 weeks later and growing for a month (Start and Gilbert 2016). Notably, herbivory has been shown to reduce the rate of successful galling (per egg laid; Cunan et al. 2015) and, in other systems, gall size. In addition to being affected by herbivory, gall size is determined by the joint effects of insect genotype, plant genotype, and environmental conditions (Cunan et al. 2015). While the complex G × G × E interactions underlying gall size in this system likely reduce heritability and dampen the response to selection (i.e., adaptive evolution), past studies have nevertheless detected putatively adaptive phenotypic responses to selection (Start and Gilbert 2016).

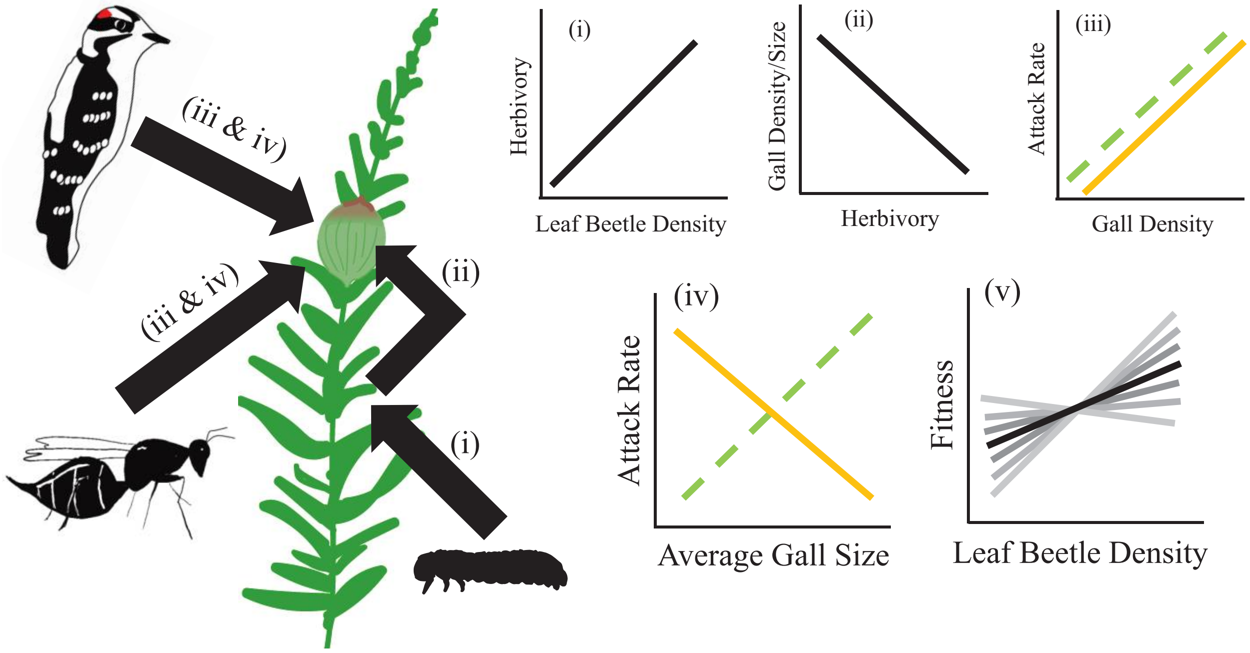

Figure 1: Diagram showing the species interactions we studied and the predictions we tested. In the left panel (illustration), *Eurosta* and goldenrod leaf beetle larvae both attack goldenrod, and *Eurosta* is itself attacked by both hymenopteran parasitoids and birds. Importantly, parasitoids and birds attack galls with opposite phenotypes, preferring small and large galls, respectively. These preferences create patterns of directional and nonlinear selection driven by the attack rates of both enemies. The graph numbers correspond to numbers next to the arrows and to the predictions presented in the final paragraph of the introduction. Prediction i: when leaf beetles are abundant, herbivory on goldenrod should increase. Prediction ii: this increased herbivory will reduce gall density (density-mediated effect) and/or gall size (trait-mediated effect). Prediction iii: assuming typical density-dependent attack rates, lower gall densities will reduce attack by both birds (dashed green line) and parasitoids (solid yellow line). Prediction iv: if herbivory causes average gall sizes to become smaller, then attack by parasitoids (solid yellow line) should increase, but attack by birds (dashed green line) should decline. Prediction v: depending on the magnitude of reduced attack rates by one or both enemies, directional and nonlinear (not pictured here) selection should shift. The exact shift in selection will then depend on the intricacies of density- and trait-mediated indirect effects. Note that the indirect effect leaf beetle → goldenrod → *Eurosta* is an example of an interaction modification, while goldenrod → *Eurosta* → bird represents an interaction chain. Overall, leaf beetle herbivory should impact the density and traits of gall makers, shifting patterns of species interactions and ultimately natural selection.

Galls are attacked by downy woodpeckers (*Dryobates pubescens*), chickadees (*Poecile atricappilus*), two species of *Eurytoma* wasp (*Eurytoma gigantea* and *Eurytoma obtusiventris*), and *Mordellistena* beetles (Uhler 1951). Of these enemies, only birds (downy woodpeckers and chickadees) and *E. gigantea* (hereafter simply referred to as *Eurytoma*) preferentially attack galls of a certain size, preferring large and small galls, respectively (Weis and Abrahamson 1985). Together, the preferences of bird predators and *Eurytoma* can cause stabilizing selection favoring *Eurosta* that produce galls of an intermediate size (Weis and Abrahamson 1985), with the optimum gall size depending on the attack rate of each (Start and Gilbert 2016; Start 2018).

Experimental Design

We used an experiment at the Koffler Scientific Reserve (KSR; http://www.ksr.utoronto.ca), Ontario, to test for the indirect effects of leaf beetles on gall size, gall density, enemy attack, and selection. In mid-May 2016, we established round plots (3.5-m radius) dominated by goldenrod. To ensure sufficiently large numbers of galls, we stocked all plots with a random mixture of ∼30 galls from the previous year (i.e., whose inhabitants were about to emerge) from around KSR. *Eurosta* emerged from these galls, mated, and then oviposited, likely increasing the abundance of galls (although they still fell well within natural densities; Start et al. 2018). Also note that while we did attempt to increase gall densities, gall makers could still have easily chosen to oviposit outside of our plots (Cronin et al. 2001). We split each plot in three by erecting 1-m-high black shade cloth, creating equal thirds of a circle. We note that the erection of barriers between sections likely caused some shading, but treatments were randomly assigned to section (see below), meaning that shading could not have driven any observed patterns. Once more, shading was likely relatively inconsequential because plants were far taller than the barriers, and the open outer edges of the plots generally provided for the penetration of light into the plots. The open edges of the plots also likely allowed for the easy dispersal of more motile herbivores (e.g., grasshoppers). Using a split-plot design (for clarity, each plot is split into three sections) allowed us to minimize local environmental differences, including differences among goldenrod genotypes (Cronin and Abrahamson 1999). After *Eurosta* had emerged and oviposited in early June, we randomly assigned each of the three sides of each plot to one of three treatments: (i) leaf beetles removed, (ii) ambient densities of leaf beetle, or (iii) double the ambient density of leaf beetles. We imposed treatments in late June by gently using a sweep net to remove beetles from the low-density treatment (i) before adding them to the high-density treatment (iii). Densities and corresponding levels of herbivory were well within the natural range, particularly in outbreak years (Cunan et al. 2015).

After establishing our plots, we estimated leaf beetle density and herbivory on five randomly selected ramets per treatment per plot biweekly for 4 weeks, at which time leaf beetles metamorphosed. Percent herbivory was estimated as the total percentage of leaf tissue missing across all leaves on each plant. Galls likely completed growing before the emergence of adult leaf beetles, so herbivory by adults was unlikely to affect gall size or selection (Cunan et al. 2015). Note that treatments were imposed after *Eurosta* oviposition but before attack by *Eurytoma* or other enemies. Because of this temporal separation, any effect of leaf beetles on *Eurosta* or its enemies must necessarily be indirect, mediated by changes in plant quality (for effects on *Eurosta*) or *Eurosta* abundance or size (for effects on enemies). Following these measurements, we allowed attack by natural enemies and hence selection to occur until April 2017. We then collected all galls, measured gall diameter, and dissected each gall to determine its content. We scored galls containing *Eurosta* larvae as survivors, those with large holes as having been killed by birds, those containing wasp larvae as having been attacked by *Eurytoma*, and empty galls showing no signs of habitation as early larval death (Abrahamson et al. 1989). Note that early larval death does not include those eggs that failed to develop galls at all. All data can be accessed in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.1sv2t4c (Start et al. 2019).

Statistical Analyses. We used a series of linear models (LMs) to test for the ecological effects of leaf beetle treatments on goldenrod, *Eurosta*, and its natural enemies. We first estimated the effect of our treatments on herbivory by using a linear mixed model (LMM), using treatment as a main effect and plot nested in sampling period as a random effect. We used the same model to estimate leaf beetle density.

We next aimed to evaluate the effects of leaf beetle herbivory on *Eurosta* and its natural enemies. We began by estimating gall density using a generalized LMM (GLMM) with a Poisson error distribution, treatment as a main effect, and plot as a random effect. We then tested for differences in gall size using an LMM with treatment and plot as main and random effects, respectively. We hypothesized that both density and gall size (trait) may affect patterns of attack and hence mortality. To test for these differences we used a series of GLMMs with binomial error distributions to separately estimate attack rates of each natural enemy and overall mortality, including treatments as main effects and plot as a random effect. We used log-likelihood ratios to test for significance of all models, with likelihoods determined from the maximum-likelihood solution.

Next, we aimed to quantify treatment-level effects on selection. We began by relativizing fitness by dividing whether a gall survived (binary variable) by the mean survival or *Eurosta* measured across all populations. We then standardized gall size by subtracting the mean gall value and dividing by the standard deviation. Relativizing and standardizing globally assumes competition among all individuals (i.e., because *Eurosta* could easily disperse between sections of each circle) and avoids errors associated with detecting differences in selection following different standardizations (De Lisle and Svensson 2017). Once more, this standardization allows us to compare treatments without confounding treatment effects with differences in standardization.

We used a series of models to estimate selection on gall size among treatments (Lande and Arnold 1983). We used separate models to estimate significance values and regression coefficients because coefficients from GLMMs cannot be directly related to selection, and LMs of transformed binary data should not be assessed for statistical significance because distribution and variance assumptions are violated (Start and Gilbert 2016; Start 2018). We began by testing for significant selection coefficients using a GLMM with a binomial error distribution. We included treatment, standardized gall size, and their interaction as main effects (a significant interaction suggests differences in selection between treatments) while controlling for plot-level differences. We then calculated selection coefficients by repeating this analysis while using an LMM. To test for differences in nonlinear selection, we repeated the above models while including the interaction of squared gall size and treatment. We then doubled the coefficient from the LMM to calculate the actual magnitude of nonlinear selection (Stinchcombe et al. 2008). We report coefficients from LMMs and significance values from GLMMs. Note that because our selection analyses use survival after enemy attack as their measure of fitness, selection caused directly by leaf beetles is null (leaf beetles never interacted directly with *Eurosta* owing to temporal mismatch), meaning that any difference in selection among treatments is equivalent to the detection of nonadditive selection in terHorst et al. (2015). Statistical analyses were conducted in R (v. 3.1.1; 2014) using the base and lme4 (Bates et al. 2015) packages.

Results

Our treatments created significant differences in herbivore density and herbivory. Relative to low-herbivore treatments, high-herbivore treatments had nine times more *Trirhabda*, with goldenrod experiencing eight times more herbivory (both $P < .001$). Low-herbivore treatments had 50% more galls ($P < .001$; fig. 2*A*), but gall size was unchanged ($P = .21$; fig. 2*B*).

Changes in herbivore density created differences in mortality and natural enemy communities. Across all treatments, 25% of *Eurosta* survived, with birds and *Eurytoma* causing mortality in 26% and 7% of *Eurosta*, respectively. Early larval death (29%) and attack by *Mordellistena* (14%) were other major sources of mortality. Beyond overall patterns of mortality, treatments differed markedly in patterns of attack. Low-herbivore treatments, where gall densities were high, experienced higher mortality rates ($P = .01$; fig. 3*A*) owing to increased attack rates of both birds and *Eurytoma* (both $P < .001$; fig. 3*B*, 3*C*), although attack by *Mordellistena* and early larval death were both unchanged ($P = .31$). Leaf beetle herbivory altered patterns of selection (gall size: $P < .001$; treatment: $P = .004$; treatment × gall size: $P = .014$). Specifically, directional selection was strongest when leaf beetles were abundant ($\beta = 0.63 \pm 0.094$ [SE]), intermediate in am-

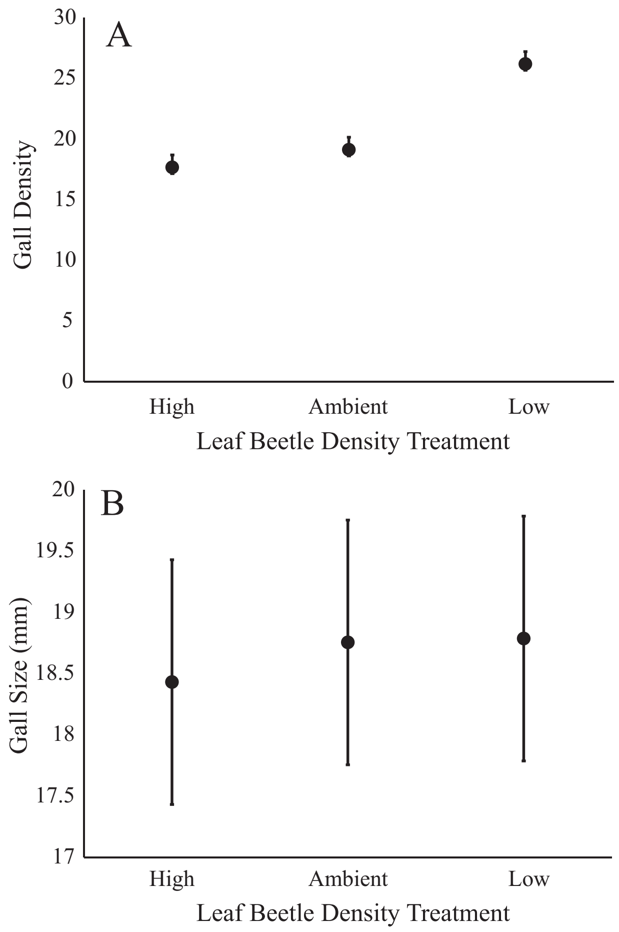

Figure 2: Gall density was greatest in treatments where leaf beetle density was high (*A*), but these same treatments had no effect on gall size (*B*). Error bars represent 95% confidence intervals.

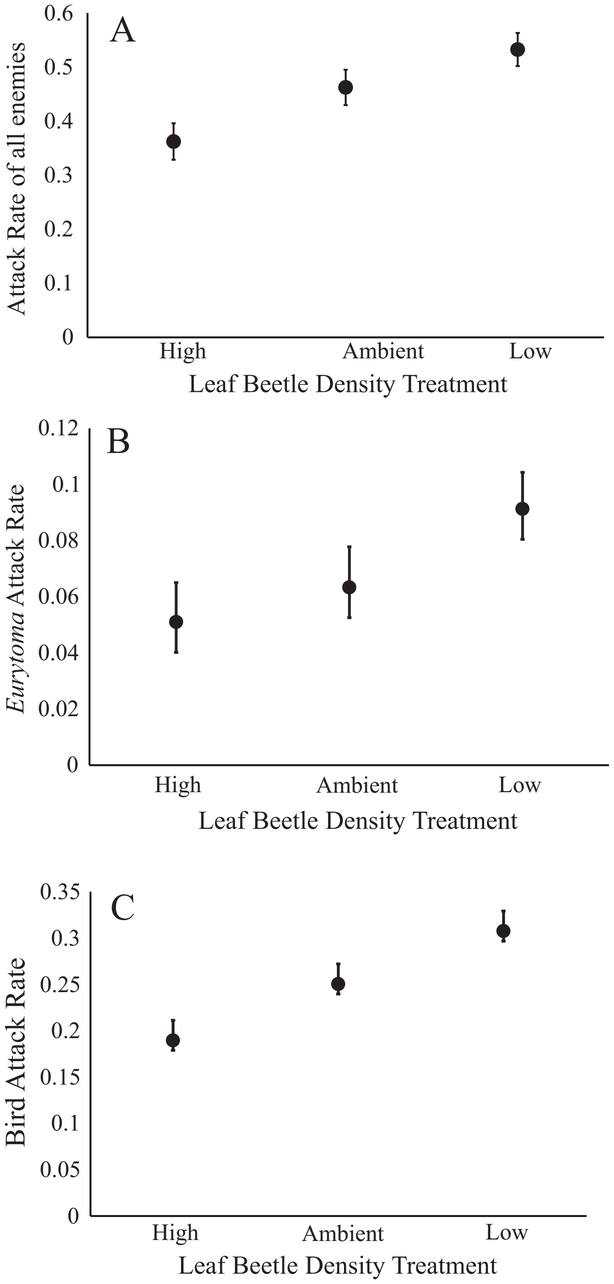

Figure 3: Patterns of mortality and enemy attack among treatments. Mortality was highest when leaf beetle density was low (*A*), with this pattern being driven by increased bird (*B*) and *Eurytoma* (*C*) attack. Error bars represent 95% confidence intervals.

bient treatments ($\beta = 0.42 \pm 0.085$ [SE]), and weakest when leaf beetles were rare ($\beta = 0.37 \pm 0.078$ [SE]; fig. 4*A*). In post hoc comparisons, selection was significantly stronger in populations where leaf beetles were abundant relative to both other treatments ($P < .01$) but did not differ between treatments with low and ambient leaf beetle abundances ($P = .68$). Conversely, nonlinear selection did not vary among treatments (treatment × gall size^2: $P = .79$) but across all treatments favored intermediate phenotypes (i.e., stabilizing selection; $\gamma = -1.35 \pm 0.35$ [SE], $P = .03$; fig. 4*B*).

Discussion

Our study demonstrates how indirect interactions can affect species traits and abundances, altering ecological (mortality, attack) and evolutionary (selection) processes. Herbivory by leaf beetles reduced gall density but had no effect on gall size (fig. 2), with this change necessarily being mediated by changes in plant resources or defense (Ohgushi 2005). Higher *Eurosta* density in low-herbivore density plots conferred higher mortality, with this effect being driven by a near doubling of *Eurytoma* attack and a 60% increase in bird attack (fig. 3). These ecological changes caused leaf beetle herbivory to strengthen directional selection without affecting nonlinear selection (fig. 4). In sum, our results show that indirect interactions mediated by changes in species traits and abundances not only are an ecological phenomenon but can equally alter patterns of selection.

Ecological indirect effects rippled through the interaction network by modifying species traits and abundances. Leaf beetles increased herbivory, likely reducing available resources or triggering induced defenses in goldenrod. As a consequence of these trait differences, leaf beetles indirectly reduced *Eurosta* abundance but had no detectable effect on gall traits (i.e., size; fig. 2). Crucially, because leaf beetles do not directly consume *Eurosta* larvae and because oviposition occurred before leaf beetle herbivory, the reduction in *Eurosta* abundance must be underlain by herbivore-induced changes in plant nutritional/defensive traits (Ohgushi 2005). Changes in plant traits are likely to be common causes of shifts in herbivore traits and abundances; trait-mediated indirect interactions are common among herbivores, likely because they rarely kill plants, but instead can induce strong defenses or changes in plant nutritional quality (Inouye and Stinchcombe 2001; Ohgushi 2005).

Beyond inferred changes in plant traits, *Eurosta* may not have differed detectably in size because many factors (i.e., fly genotype, plant genotype, other environmental differences) influence gall size, causing large differences in gall size within treatments (Cronin and Abrahamson 1999). Put simply, the many other factors influencing phenotype in this system may have overwhelmed any effect of reduced plant quality driven by leaf beetle herbivory. Instead, galls were 1.5 times more abundant when leaf beetles were rare, with this effect potentially mediated by either (i) choice of oviposition site (Cronin and Abrahamson 2001) or (ii) differences in *Eurosta* mortality rates prior to gall formation (Cunan et al. 2015). In our study, herbivory treatments were imposed after oviposition, precluding the former mechanism. Instead, consistent with past studies in the same system (Cunan et al. 2015), reduced gall density was likely caused by a high level of mortality prior to gall formation on highly herbivorized plants. Overall, interaction modifications can cause differences in traits, abundances, or both. Generally, it may be the case that trait differences are more common when the trait in question is particularly plastic. Conversely, abundance differences may arise when organisms are already near the minimum condition required for survival or when inducible differences in plants are so large as to cause high levels of mortality. Whether traits, abundances, or both are modified then has downstream consequences for species interactions and selection.

Abundance differences caused large changes in the strength of species interactions in the interaction chain. Specifically, leaf beetle–mediated differences in *Eurosta* abundance created divergent patterns of attack by natural enemies. *Eurytoma* and bird attack increased by 1.8 and 1.6 times, respectively, causing an 8% increase in overall mortality (fig. 3). These findings are consistent with an expansive literature, theoretical and empirical, showing that consumers preferentially forage in areas where resources are dense (Holling 1959; Hassell 1978; Berryman 1992; Abrams and Ginzburg 2000). Density dependence has also been tested and found reasonable support among gall makers and their enemies, although tests in this system have either failed to detect density dependence (Weis and Kapelinski 1994) or found density dependence in some but not all enemies (Cappuccino 1992). Regardless, despite the apparent commonality of density-dependent attack, virtually all examples of diffuse coevolution cite trait rather than density differences as underlying shifts in selection (Janzen 1980; Pilson 1996; Inouye and Stinchcombe 2001; Stinchcombe and Rausher 2001; terHorst 2015). The surprising lack of described density-mediated mechanisms may arise, however, because of (1) the large focus on plants that are rarely killed by their enemies and (2) a more thorough treatment of interaction modification versus interaction chains. Interestingly, the density-mediated mechanism found here shows that while leaf beetle herbivory may increase mortality prior to gall formation, this difference may be partially compensated for by decreasing consumer-induced mortality. Such tradeoffs may be common when indirect interactions reduce abundance of the focal species (negative indirect effect), but by lowering density they also reduce enemy attack (positive indirect effect). Alternatively, negative indirect effects of one species on another may increase enemy attack if it results in reduced defenses rather than reduced abundance (Inouye and Stinchcombe 2001), a pattern that may be common, for

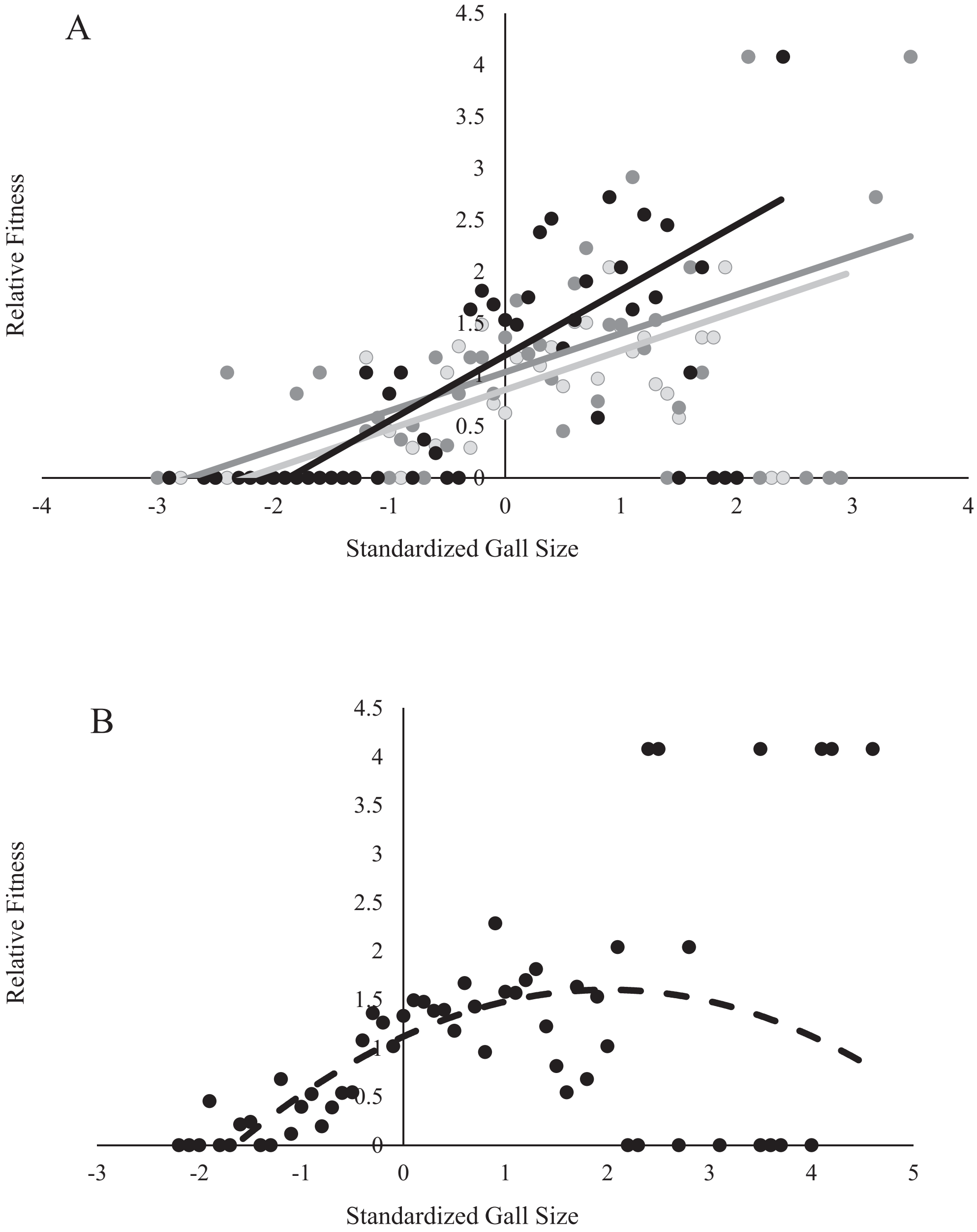

Figure 4: Indirect ecological effects drove divergent patterns of selection among treatments. *A*, Directional selection differed in strength among treatments. Directional selection was strongest when leaf beetles were abundant (black circles), intermediate when at ambient levels (dark gray circles), and weak when leaf beetles were rare (light gray circles). We do note, however, that ambient and low-abundance treatments did not create selections that were significantly different from one another. *B*, Nonlinear selection did not differ among treatments but across all treatments favored intermediate phenotypes (stabilizing selection). As such, all data have been plotted together (as denoted by the dashed line). Note that the optimum value (the highest point on the dashed line in *B*) is well to the right of the average gall size, further demonstrating that directional selection should favor larger galls. Points show mean relative fitness for groups of galls binned in standardized gall sizes of 0.1, but the best-fit lines show estimated values from linear mixed models fit with the original binomial data set. Points that lie along the *X*-axis or at ∼4 represent bins with only one or two galls (notice that these points are at the phenotypic extremes) and therefore have a weaker effect on patterns of selection.

example, among immune-modulating pathogens (Graham 2008). Broadly, negative indirect effects of one species on another may have negative or positive effects on their enemies depending on whether such effects are mediated by reduced abundance or reduced antipredator/antipathogen defenses.

Indirect ecological interactions are common among plants, herbivores, and their enemies (Ohgushi 2005). We extend this pattern to show that these ecological effects ultimately create differences in selection among populations and multispecies communities (terHorst et al. 2015). While directional selection always favored larger galls, selection in populations where leaf beetles were rare was only 60% as strong as in high-density plots (fig. 4). These evolutionary cold spots were created by the shifting balance of attack rates by enemies preferring opposite phenotypes. When leaf beetles were rare and galls common, attack by both enemies increased but the absolute increase in bird attack rate was larger (fig. 3), reducing the strength of selection favoring large galls (fig. 4). Complex patterns of density-dependent selection are likely to be common to the many systems where resource species experience a trade-off (Sih et al. 1985; Weis and Abrahamson 1985; Lively 1986; Chase et al. 2002; Relyea 2002), balancing investing in defense against one or another enemy, and where those enemies are differently affected by an indirect interaction (e.g., different density-dependent attack functions). In short, the effect of indirect effects on enemies will ultimately create changes in selection for antipredator traits and those traits against which they trade off.

When aiming to describe and understand patterns of selection, how important is it to understand the complex interactions inherent to multispecies communities? This question is certainly outside the scope of the current article (but for a start, see terHorst et al. 2018), but we can address some of what we feel are the key concepts underlying the answer to this question. First, complex interactions are likely to be important when the change in any one organism ripples strongly and distantly through the associated interaction network. Rippling interactions may be more likely in simple interaction networks with stronger interactions, a pattern echoed by the greater strength of indirect effects in aquatic systems where species interactions tend to cause larger changes in density (Borer et al. 2006). Beyond characteristics of whole communities, multispecies interactions may be more important for understanding selection within communities when considering changes in a particularly important species. For example, changes in leaf beetle herbivory had a large effect on selection, but changing the abundance of a less impactful herbivore (e.g., pea aphids) may not affect selection on gall size. Put simply, complex indirect interactions will affect selection when those interactions are strong. Finally, we must understand the degree to which interactions influence traits that structure selection (e.g., the covariance between herbivory and gall size). When this covariance is high, relatively small changes in interaction strength can result in large differences in selection. The effects of complex indirect interactions on selection will then depend on the interaction between (1) the strength of those indirect effects and (2) the sensitivity of traits to species interactions.

Selection in multispecies communities or otherwise can in some cases cause local adaptation and eco-evolutionary feedbacks. In the simplest case, in our study we would expect communities with perennially high leaf beetle densities to drive the evolution of *Eurosta* populations with relatively large galls (large positive selection coefficients; fig. 4). However, the complex interactions underlying gall size are likely to slow any response to selection, and the spatial scale at which our study was conducted is unlikely to foster local adaptation because all species are capable of dispersing over far larger scales (Cronin et al. 2001). Nonetheless, differences in the frequency of patches experiencing high leaf beetle herbivory between regions may cause adaptation at broader scales. Beyond the limitations of our study, local adaptation may not occur for a multitude of reasons (e.g., variable selection, overwhelming gene flow), and local adaptation driven by indirect effects may be further complicated (Inouye and Stinchcombe 2001; terHorst et al. 2015). For example, the above-stated simplest case for our study assumes that (1) plants do not evolve to become less attacked or impacted by leaf beetles and (2) *Eurosta* does not evolve to become more tolerant of poor host plant quality (i.e., evolution of a G × E interaction). The likelihood of local adaptation and the nature of any eco-evolutionary feedback will then depend on which species along the chain of interactions evolve and in what way they evolve (e.g., to enemies or host quality) in response to leaf beetle herbivory. The potential evolution of myriad species in interactions webs through direct and indirect effects distinguishes evolutionary indirect effects from simple coevolution and necessitates the development of novel approaches (terHorst et al. 2018). More generally, we suggest that indirect effects are liable to create simultaneous selection in many species (populations) in an interaction web (terHorst et al. 2015; McPeek 2017) and that we must understand the independent and joint effects of this selection to predict the eco-evolutionary dynamics of complex communities.

By incorporating ecological indirect effects with evolutionary selection in a well-understood system, our study aimed to improve our understanding of selection in complex multispecies communities (terHorst 2018). We show that, consistent with past work, indirect ecological effects can ripple across interaction webs by changing either species densities or traits (fig. 2; Shurin et al. 2002; Ohgushi 2005). These shifts in species interactions and community composition (fig. 3) can ultimately create complex but predictable shifts in selection (fig. 4; terHorst et al. 2015, McPeek 2017). However, we caution that while indirect effects could cause local adaptation, this may be unlikely given the complexity of multiple evolving species and traits in diverse interaction networks (Inouye and Stinchcombe 2001; terHorst et al. 2015; McPeek 2017). We suggest that an understanding of selection in complex multispecies communities will facilitate the rapprochement of community ecology and evolution, garnering insights for both fields.

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Eurosta galls on tall goldenrod. Galls differ in size and density, shaping patterns of enemy attack. Changing patterns of attack are ultimately responsible for creating differences in natural selection among populations. Photo credit: Tobias David Mankis.