Phenotypic evolution is more constrained in simpler food webs

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Abstract

Global change is simplifying the structure of ecological networks; however, we are currently in a poor position to predict how these simplified communities will affect the evolutionary potential of remaining populations. Theory on adaptive landscapes provides a framework for predicting how selection constrains phenotypic evolution, but often treats the community context of evolving populations as a "black box". Here, we integrate ecological networks and adaptive landscapes to examine how changes in food-web complexity shape evolutionary constraints. We conducted a field experiment that manipulated the diversity of insect parasitoids (food-web complexity) that were able to impose selection on an insect herbivore. We then measured herbivore survival as a function of three key phenotypic traits. We found that more traits were under selection in simpler vs. more complex food webs. The adaptive landscape was more neutral in complex food webs because different parasitoid species impose different selection pressures, minimizing relative fitness differences among phenotypes. Our results suggest that phenotypic evolution becomes more constrained in simplified food webs. This indicates that the simplification of ecological communities may constrain the adaptive potential of remaining populations to future environmental change.

"What escapes the eye, however, is a much more insidious kind of extinction: the extinction of ecological interactions." Janzen (1974)

Introduction

The adaptive landscape provides a powerful framework for understanding how natural selection has shaped the evolution of biodiversity —from genes to phenotypes to species (Wright, 1931; Simpson, 1944; Arnold et al., 2001). More than a metaphor, the adaptive landscape links quantitative genetic and phenotypic variation to evolution by natural selection (Lande, 1979; Arnold and Wade, 1984*a*,*b*). Ecological interactions often play a key role in shaping adaptive landscapes, as evidenced by the role of antagonistic (Schluter, 2000; Abrams, 2000) and mutualistic (Bronstein et al., 2006) interactions in driving evolutionary change. Although there is clear evidence that pairwise interactions can shape the adaptive landscape, we still have a poor understanding of how the adaptive landscape is shaped by the community context (McPeek, 2017; terHorst et al., 2018). Resolution on how the community context shapes phenotypic evolution is urgently needed though, given the rapid impacts of climate change on ecological communities (Scheffers et al., 2016).

Ecological networks, such as food webs describing who-eats-whom in ecological communities, provide an explicit representation of the community context (Bascompte and Jordano, 2014; McCann, 2012). Here, we integrate ecological networks and adaptive landscapes to understand how the community context constrains evolutionary change (Arnold, 1992). Different aspects of evolutionary constraints can be inferred by quantifying the slope and curvature of the adaptive landscape. For example, the slope is determined by directional selection gradients acting on each phenotypic trait and influences the trajectory of evolutionary change (Lande, 1979; Arnold, 1992). Evolutionary trajectories become more constrained with an increase in the number of traits under selection, as this diminishes the number of optimal solutions (Arnold, 2003). The curvature of the adaptive landscape can also constrain evolution through its indirect effect on genetic constraints (Arnold,

1992; Hansen and Houle, 2008). Genetic constraints are largely governed by a population's **G**-matrix —the additive genetic variances and covariances between traits (Hansen and Houle, 2008). In general, genetic constraints will increase with the number of traits under directional or stabilizing selection, as this will decrease the additive genetic variance in those traits (Hansen and Houle, 2008). Genetic constraints may also increase with the number of trait combinations under correlational selection, as this type of selection decreases the evolutionary independence of traits (Hansen and Houle, 2008). If we want to predict how the community context constrains evolutionary change, we must understand how ecological networks shape the adaptive landscape.

Global change is simplifying the structure of ecological communities, which may influence evolutionary constraints in a number of ways. For example, in a multitrophic community or food web, if different consumers impose directional selection on different traits of a shared resource, then more complex food webs may constrain evolution by increasing the number of traits under selection. Alternatively, if consumers impose selection on different values of a trait, then their selective effects would cancel each other out in more complex food webs. To examine these different possibilities, we conducted a field experiment that manipulated the complexity of trophic interactions (hereafter food-web complexity) associated with an abundant insect herbivore (*Iteomyia salicisverruca*, Family Cecidomyiidae; fig. 1). The larvae of this herbivore induce tooth-shaped galls when they feed on the developing leaves of willow trees (*Salix* sp., Russo, 2006). These galls protect larva from attack by generalist predators (e.g. ants, spiders), but they suffer high mortality from egg and larval parasitoids (Barbour et al., 2016). We manipulated food-web complexity by either excluding the guild of larval parasitoids (simple food web) or allowing both egg and larval parasitoids to impose selection on gall midge traits (complex food web; fig. 1). We then applied modern statistical methods to quantify the effect of food-web complexity on the slope and curvature of the gall midge's adaptive landscape. Taken together, our study gives insight to how local extinctions, and concomitant decreases in food-web complexity, may constrain the evolution of interacting populations.

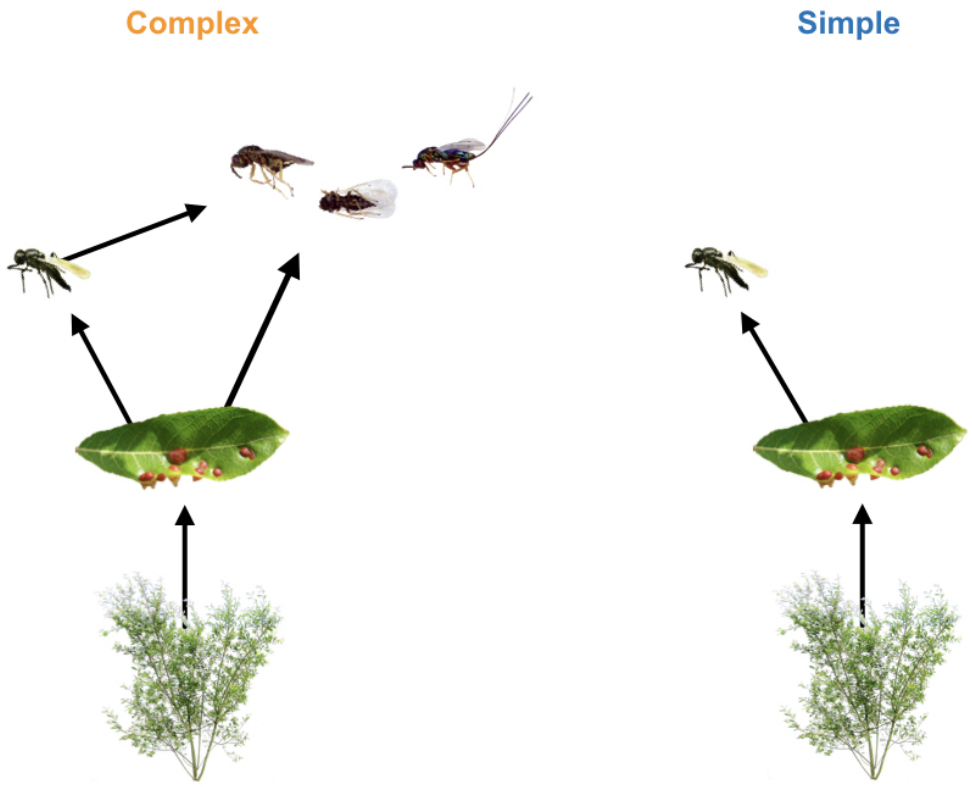

Figure 1: Experimental manipulation of food-web complexity associated with a leaf-galling midge (*Iteomyia salicisverruca*) feeding on the willow *Salix hookeriana*. Black arrows denote the flow of energy in this network of trophic interactions. In the complex food-web treatment, we allowed the full suite of egg and larval parasitoids to impose selection. In the simple food-web treatment, we used mesh bags to exclude the guild of larval parasitoids, only allowing the egg parasitoid (*Platygaster* sp.) to impose selection. Larval parasitoids include the following species (from left to right): *Mesopolobus* sp. (Family: Pteromalidae); *Tetrastichus* sp. (Family: Eulophidae); and *Torymus* sp. (Family: Torymidae).

Methods

Study Site

We conducted our study within a four-year old common garden experiment of coastal willow (*Salix hookeriana*) located at Humboldt Bay National Wildlife Refuge (HBNWR) (40 40'53"N, 124 12'4"W) near Loleta, California, USA. This common garden consists of 26 different willow genotypes that were collected from a single population of willows growing around Humboldt Bay. Stem cuttings of each genotype (25 replicates per genotype) were planted in a completely randomized design in two hectares of a former cattle pasture at HBNWR. Willows at our study site begin flowering in February and reach their peak growth in early August. During this study, willows had reached 5 - 9m in height. Further details on the genotyping and planting of the common garden are available in Barbour et al. (2015).

Manipulating Food-web Complexity

We setup our food-web manipulation across 128 plants soon after galls began developing on willows in early June of 2013. These 128 plants came from eight different plant genotypes that spanned the range of trait variation observed in this willow population (Barbour et al., 2015). For the complex food-web treatment (eight replicates per genotype), we used flagging tape to mark 14 galled leaves per plant (~30 larvae), allowing the full suite of egg and larval parasitoids to impose selection. Marking galls with flagging tape ensured that we compared control and treatment galls with similar phenology when we collected galls later in the season. For the simple food-web treatment, we enclosed 14 galled leaves with 10x15cm organza bags (ULINE, Pleasant Prairie, WI, USA) to exclude three parasitoid species that attack during larval development. This treatment did not exclude the egg parasitoid *Platygaster* sp., which attacks prior to gall initiation (larva initiate gall development in Cecidomyiid midges: Gagné, 1989). In late August, we collected marked and bagged galls from each plant, placed them into 30 mL vials and kept them in the lab for 4

months at room temperature. We then opened galls under a dissecting scope and determined whether larvae survived to pupation (our measure of fitness) or were parasitized. Since we were interested in selection imposed by parasitoids, we excluded unknown sources of mortality. For the food-web treatment that excluded larval parasitoids (simple food web), we further restricted our data by removing any incidental instances of parasitism by a larval parasitoid. This represented less than 3% of the observations in this food-web treatment and allowed us to focus our inferences of selection on those imposed by the egg parasitoid. Our final dataset contains survival estimates for 1285 larvae from 613 galls and 111 plants.

Measuring Phenotypic Traits

We collected data on three different traits that we expected to influence larval survival based on our previous work (Barbour et al., 2016) and other work with gall midges (Weis et al., 1983; Heath et al., 2018). First, we measured gall diameter as the size of each gall chamber to the nearest 0.01 mm at its maximum diameter (perpendicular to the direction of plant tissue growth). Our previous work has shown that larger galls are associated with higher survival (Barbour et al., 2016). Second, we measured clutch size by counting the number of chambers in each gall (Weis et al., 1983; Heath et al., 2018). All larvae collected from the same multi-chambered gall were scored with the same clutch size. Third, we measured oviposition preference as the density of larvae observed on a plant in an independent survey. We did this by randomly sampling five branches per tree and counting the number of individual gall chambers (number of larvae). We then converted these counts to a measure of larval density per 100 shoots by counting the number of shoots on the last branch we sampled. All larvae collected from the same plant were scored with the same oviposition preference. Measuring larval densities on plants in the field is a common method for measuring oviposition preference (Gripenberg et al., 2010); however, caution must be taken in inferring 'preference' as larval densities can be influenced by processes other than preference (Singer, 1986). Fortunately, a couple features of our study system suggest that larval densities may

be a good proxy for oviposition preference. For example, since our data comes from a randomized placement of plant genotypes in a common garden, there is no consistent bias in which plant genotypes females are exposed to while searching for oviposition sites. Also, egg predation is a minor source of mortality for galling insects in general (Hawkins et al., 1997); therefore, we do not expect any prior egg predation to bias our estimates of observed larval densities.

Quantifying the Adaptive Landscape

Our analysis consisted of three parts. First, we used generalized linear mixed models (GLMM) to quantify selection surfaces —linear and nonlinear relationships between absolute fitness (W) and phenotypic traits (z_i) of individuals —in each food-web treatment. Second, we translated selection surfaces into the scale of relative fitness (w) in order to calculate selection gradients. Third, we used our estimates of selection gradients to characterize the slope and curvature of the adaptive landscape. Note that inferring adaptive landscapes from selection surfaces assumes that trait distributions are multivariate normal (Lande and Arnold, 1983). To approximate this assumption, we log-transformed clutch size and square-root transformed oviposition preference. We then scaled all phenotypic traits (mean=0 and SD=1) in order to calculate standardized selection gradients that were comparable across traits and with other studies of natural selection.

Selection surface: Since larval survival was our measure of absolute fitness, we used a GLMM that assumed a binomial error distribution (and logit-link function). To approximate the selection surface, we modelled larval survival as a function of food-web treatment as well as linear (α_{z_i}), quadratic ($\alpha_{z_i:z_i}$), and linear interactions ($\alpha_{z_i:z_j}$) between each trait. We also allowed these trait-fitness relationships (α) to vary between food-web treatments. Note that to obtain valid estimates of linear trait-fitness relationships, we removed nonlinear terms prior to estimating linear relationships (Lande and Arnold, 1983). Other approaches have been advocated for approximating selection surfaces (Schluter, 1988); however, our approach enables us to calculate selection gradients, and thus is more appropriate for approximating the adaptive landscape (Arnold, 2003). To account for

the nonindependence of clutch size (measured at gall level) and oviposition preference (measured at plant level) as well as any independent effects of willow genotype on larval survival, we modelled gall ID nested within plant ID nested within genotype ID as random effects. Although statistical models with random effects are not common in analyses of natural selection, we think that modelling random effects can mitigate biased estimates of selection due to environmental covariances between traits and fitness (Rausher, 1992). Since our end goal was to characterize the relationship between mean trait values and mean fitness (adaptive landscape), we assumed the mean value of our random effects (i.e., setting them to zero) when calculating trait-fitness relationships. We then used parametric bootstrapping (1,000 replicates) to estimate the effect of food-web treatment on larval survival as well as trait-fitness relationships in each food-web treatment. To determine whether trait-fitness relationships differed between food-web treatments, we calculated the difference in bootstrapped replicates between treatments.

Selection gradients: We used the method of Janzen and Stern (1998) to translate trait-fitness relationships (α) into the scale of relative fitness in order to calculate directional (β_{z_i}), quadratic ($\gamma_{z_i:z_i}$), and correlational ($\gamma_{z_i:z_j}$) selection gradients. Briefly, this method calculates the average gradient of selection surfaces by multiplying the average of $W(z)[1 - W(z)]$ by each regression coefficient (e.g. α_{z_i}, $\alpha_{z_i:z_i}$, or $\alpha_{z_i:z_j}$). We then divided this average gradient by mean fitness ($\bar{W}$) to put it on the scale of relative fitness, and thus interpretable as a selection gradient. We estimated selection gradients separately for each food-web treatment. Note that we doubled all quadratic terms prior to calculating selection gradients to put them on the same scale as estimates of directional and correlational selection (Stinchcombe et al., 2008).

Evolutionary constraints: We quantified the effects of food-web complexity on evolutionary constraints by inspecting the slope and curvature of the adaptive landscape. The number of selective constraints is determined by the slope of the adaptive landscape, which in our study corresponds to:

$$\text{Slope} = \beta = \begin{pmatrix} \beta_{\text{Diam}} \\ \beta_{\text{Clutch}} \\ \beta_{\text{Pref}} \end{pmatrix}$$

By comparing the number of traits that determine the slope (i.e., nonzero directional selection gradients) in simpler vs. more complex food webs, we can infer the effect of food-web complexity on selective constraints.

The indirect effects of selection on genetic constraints is governed by the curvature of the adaptive landscape ($\text{C} = \gamma - \beta\beta^{\text{T}}$), which in our study corresponds to:

$$\mathbf{C} = \begin{pmatrix} \gamma_{\text{Diam:Diam}} & & \\ \gamma_{\text{Clutch:Diam}} & \gamma_{\text{Clutch:Clutch}} & \\ \gamma_{\text{Pref:Diam}} & \gamma_{\text{Pref:Clutch}} & \gamma_{\text{Pref:Pref}} \end{pmatrix} - \begin{pmatrix} \beta_{\text{Diam}}\beta_{\text{Diam}} & & \\ \beta_{\text{Clutch}}\beta_{\text{Diam}} & \beta_{\text{Clutch}}\beta_{\text{Clutch}} & \\ \beta_{\text{Pref}}\beta_{\text{Diam}} & \beta_{\text{Pref}}\beta_{\text{Clutch}} & \beta_{\text{Pref}}\beta_{\text{Pref}} \end{pmatrix}$$

$$\mathbf{C} = \begin{pmatrix} C_{\text{Diam:Diam}} & & \\ C_{\text{Clutch:Diam}} & C_{\text{Clutch:Clutch}} & \\ C_{\text{Pref:Diam}} & C_{\text{Pref:Clutch}} & C_{\text{Pref:Pref}} \end{pmatrix}$$

Note that we ommitted the upper triangle of each matrix for clarity since it is simply the reflection of the lower triangle. The sign of diagonal terms of the curvature matrix dictates whether selection will increase (+), decrease (-), or cause no change (0) in the additive genetic variance of a trait. Similarly, any nonzero covariance terms (off-diagonal) are indicative of selection for trait integration (i.e., less independence). Therefore, we can infer the indirect effects of selection on genetic constraints by counting the number of negative signs along the diagonal (decrease in additive genetic variance) and the number of nonzero terms along the off-diagonal (trait integration) of the curvature matrix.

Adjusting for biased measurements of selection

Rather than imposing selection, parasitoids may influence the expression of herbivore traits which could bias measurements of selection. In our system, it was plausible that parasitoids may influence chamber diameter by altering larval feeding behavior or killing larvae before they complete their development. To estimate this potential bias, we subset our data to only include galls where there was variation in larval survival within the same gall (i.e. 1 > mean survival > 0). If we assume that larvae within each gall should have similar chamber diameters because they come from the same clutch and experience the same local environment (an assumption our data supports: gall ID explains 54% of the variance in chamber diameter), then the relationship between chamber diameter and larval survival in this data subset represents the effect of parasitism on trait expression (i.e. bias). We used a GLMM with the same structure as described above except that we modelled only a linear relationship between chamber diameter and larval survival (α_{Diam}). We detected a positive bias in both food-web treatments (complex α_{Diam}= 0.36 [0.05, 0.67]; simple α_{Diam}= 0.42 [0.01, 0.82]), indicating that unadjusted relationships would overestimate the strength of selection on chamber diameter. To account for this bias, we substracted our mean estimates of bias from our estimates with the full dataset prior to calculating chamber diameter's trait-fitness relationship and directional selection gradient.

Measuring selection on the extended phenotype of egg parasitoids

Once parasitized, the gall phenotype becomes the extended phenotype of the egg parasitoid. This extended phenotype may influence the egg parasitoid's survival in the face of larval parasitoids, and thus experiences selection. Our food-web manipulation allows us to measure selection imposed by larval parasitoids on the extended phenotype of egg parasitoids. Using the same models as described above, we substituted egg parasitism as our response variable to quantify trait-fitness relationships and selection gradients acting on the egg parasitoid. Note that we cannot test the effect of food-web complexity on the egg parasitoid's adaptive landscape —we can only estimate

the selection imposed by larval parasitoids. This comparison is still useful though in determining the extent to which the community context may have indirect evolutionary effects by altering selection on multiple interacting populations.

All analyses and visualizations were conducted in R (R Core Team, 2018). Unless otherwise noted, we report mean estimates of trait-fitness relationships and selection gradients with 95% confidence intervals in brackets. Note that for visualizing the adaptive landscape we restrict trait axes to ± 1 SD of the mean trait value. This emphasizes the fact that we can only reliably estimate the shape of the adaptive landscape near the mean phenotype of the population (Arnold et al., 2001). We also plot mean larval survival on a natural log scale to accurately reflect the mathematical definition of the adaptive landscape (Arnold, 2003). All data and code to reproduce the reported results are publically available on GitHub (https://github.com/mabarbour/complexity_selection) and have been archived on Zenodo (https://zenodo.org/badge/latestdoi/108833263).

Results

Simpler food webs increase selective constraints

We found that more gall midge traits experienced directional selection in the simple (3 of 3) vs. complex food-web treatment (1 of 3)(table 1). For example, we observed directional selection for smaller clutch sizes in the simple food web, but there was no evidence of selection acting on this trait in the complex food web (table 1; fig. 2C). This absence of selection appeared to be a result of conflicting selection pressures imposed by each guild of parasitoids. Specifically, when we subset our data to focus on differences between parasitoid guilds, we found that larval parasitoids actually impose directional selection for larger clutch sizes (β_{Clutch}= 0.13 [0.04, 0.24]). In the simple food web, we also observed clear evidence of directional selection for midges to avoid ovipositing on plants with high densities of conspecifics (table 1; fig. 2B); however, this relationship was weaker in complex food webs (table 1). This was likely a result of larval parasitoids imposing greater

mortality on egg parasitoids at high gall midge densities (see Selection on the extended phenotype of egg parasitoids section), and thus a less than additive effect on gall midges. Chamber diameter experienced positive directional selection in both food-web treatments, but selection was 66% higher in complex food webs (table 1; fig. 2). This was not due to any difference between egg and larval parasitoids (β_{Diam} contrast = 0.01 [0.15, -0.11]), but was due to the lower average survival in complex food webs ($\bar{W}$ contrast -0.27 [-0.11, -0.42]).

Table 1: Standardized selection gradients acting on gall midges in complex vs. simple food webs.

Selection gradient	**Complex**	**Simple**	**Contrast**
β_{Diam}	**0.34 [0.22, 0.48]**	**0.21 [0.12, 0.31]**	**0.14 [0.27, 0]**
β_{Clutch}	0.06 [-0.05, 0.17]	**-0.09 [-0.17, -0.01]**	**0.15 [0.29, 0.03]**
β_{Pref}	-0.13 [-0.29, 0.05]	**-0.16 [-0.26, -0.06]**	0.03 [0.21, -0.15]
$\gamma_{\text{Diam,Diam}}$	0.13 [-0.06, 0.33]	0.1 [-0.02, 0.23]	0.03 [0.27, -0.2]
$\gamma_{\text{Clutch,Clutch}}$	-0.05 [-0.27, 0.18]	-0.11 [-0.28, 0.03]	0.06 [0.32, -0.2]
$\gamma_{\text{Pref,Pref}}$	**0.34 [0.07, 0.63]**	0.02 [-0.14, 0.18]	**0.32 [0.64, 0.01]**
$\gamma_{\text{Diam,Clutch}}$	-0.04 [-0.16, 0.08]	-0.07 [-0.15, 0.02]	0.02 [0.17, -0.12]
$\gamma_{\text{Diam,Pref}}$	-0.13 [-0.29, 0.02]	-0.02 [-0.1, 0.07]	-0.12 [0.05, -0.3]
$\gamma_{\text{Clutch,Pref}}$	0.03 [-0.1, 0.18]	0 [-0.07, 0.07]	0.03 [0.18, -0.12]

Note: Values in brackets represent 95% confidence intervals. Bold values indicate that the 95% CI does not overlap zero. β_{Diam} has been adjusted for bias.

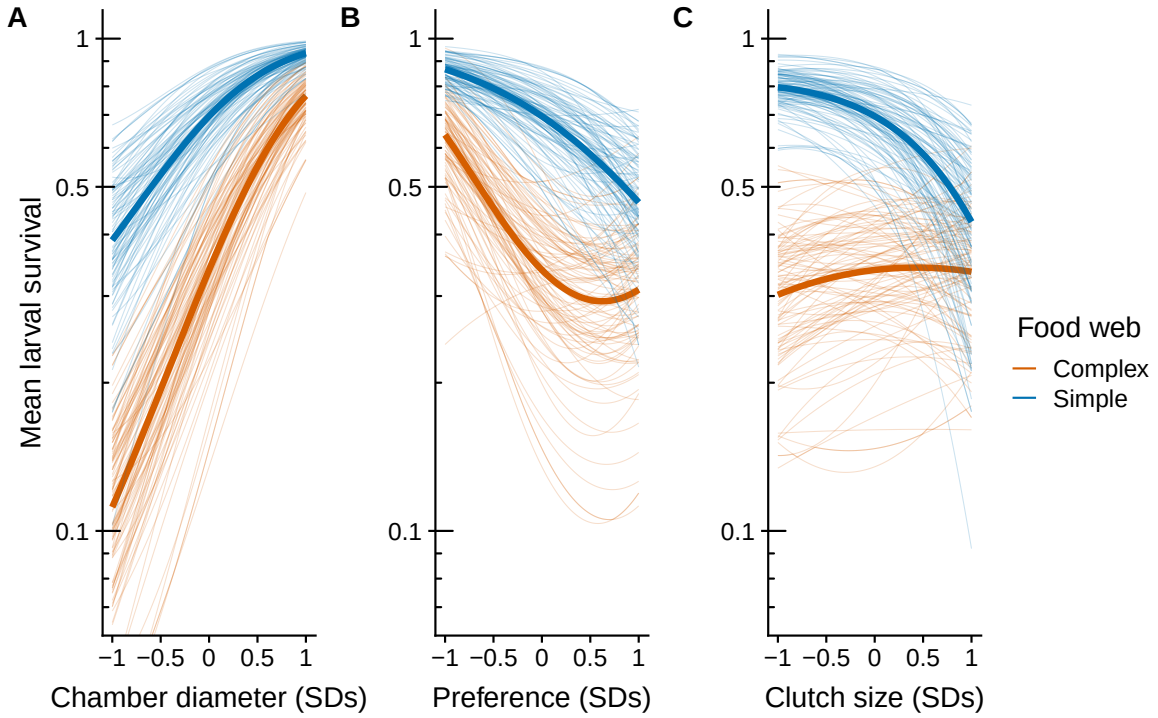

Figure 2: Adaptive landscape of gall midge phenotypes in complex vs. simple food webs. Each panel corresponds to a different phenotypic trait: chamber diameter (A); oviposition preference (B); and clutch size (C). Bold lines represent selection experienced in complex (orange) and simple (blue) food webs. Thin lines represent bootstrapped replicates to show the uncertainty in selection. For clarity, we only display 100 bootstraps even though inferences are based on 1,000 replicates. Note that mean larval survival is plotted on a natural log scale to reflect the mathematical defintion of the adaptive landscape.

Simpler food webs increase genetic constraints

The curvature of the adaptive landscape indirectly affects genetic constraints and is influenced by directional, quadratic, and correlational selection gradients. Our food-web treatment did not alter correlational selection for any combination of traits (table 1). Similarly, our food-web treatment

did not influence quadratic selection on either chamber diameter or clutch size (table 1; fig. 2A,C). In contrast, our food-web treatment did alter quadratic selection acting on oviposition preference (table 1). The negative relationship between oviposition preference and larval survival dampened at high densities in complex, but not simple food webs (fig. 2B). This dampened relationship was partly due to a trend for nonlinear selection imposed by larval parasitoids ($\gamma_{\text{Pref:Pref}}$= 0.18 [-0.02, 0.42]), but was also magnified by the lower average survival in complex food webs.

To estimate the net effect of food-web complexity on the curvature (C) of the adaptive landscape, we only retained nonzero selection gradients (i.e. 95% CI did not overlap zero) prior to calculating the curvature ($\gamma - \beta\beta^{\text{T}}$) in each food-web treatment. We found that the curvatures of the adaptive landscape exhibited the following structures:

$$\mathbf{C} = \begin{pmatrix} C_{\text{Diam:Diam}} & & \\ C_{\text{Clutch:Diam}} & C_{\text{Clutch:Clutch}} & \\ C_{\text{Pref:Diam}} & C_{\text{Pref:Clutch}} & C_{\text{Pref:Pref}} \end{pmatrix}$$

$$\mathbf{C}_{\text{Complex}} = \begin{pmatrix} -0.12 & & \\ 0 & 0 & \\ 0 & 0 & 0.34 \end{pmatrix}$$

$$\mathbf{C}_{\text{Simple}} = \begin{pmatrix} -0.04 & & \\ 0.02 & -0.01 & \\ 0.03 & -0.01 & -0.03 \end{pmatrix}$$

Remember that we can infer the indirect effects of selection on genetic constraints by counting the number of negative signs along the diagonal (decrease in additive genetic variance) and the number of nonzero terms along the off-diagonal (trait integration) of the curvature matrix. The structure of these matrices indicates that there are more selective constraints imposed on the gall midge in the simpler (6 of 6) vs. more complex (1 of 6) food web. Simpler food webs acted to decrease genetic

variance for all three phenotypic traits (negative diagonal terms), whereas only one trait (chamber diameter) experienced a decrease in additive genetic variance in the more complex food web. For genetic covariances, the simpler food web favored integration among all three phenotypic traits (nonzero off-diagonal terms), and thus constraints along all three axes of covariance (fig. 3). In contrast, there was no strong evidence of trait integration in the more complex food web (fig. 3).

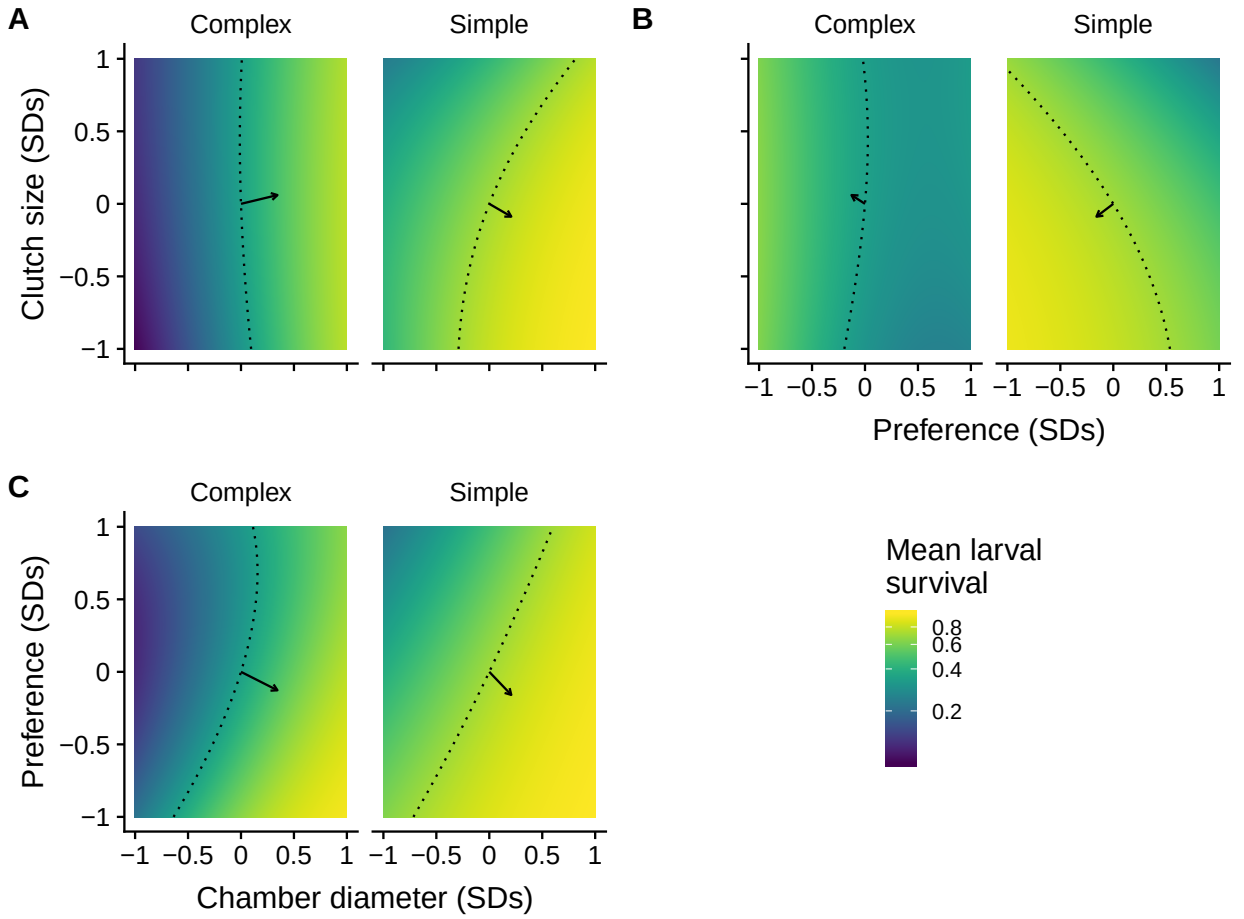

Figure 3: Two dimensional view of adaptive landscapes of gall midge phenotypes in complex vs. simple food webs. Each panel corresponds to a different combination of phenotypic traits: clutch size and chamber diameter (A); clutch size and oviposition preference (B); oviposition preference and chamber diameter (C). Arrows represent mean estimates of directional selection gradients, while contours represent predicted larval survival of the mean phenotype in each food-web treatment. Notice that arrows point more toward a corner of the adaptive landscape for each combination of traits in the simple vs. complex food-web treatment. This indicates that trait integration (covariance) is more strongly favored in simpler vs. more complex food webs. Note that mean larval survival is plotted on a natural log scale to reflect the mathematical definition of the adaptive landscape.

Selection on the extended phenotype of egg parasitoids

Only gall midge preference influenced the probability of observing egg parasitoids in complex vs. simple food webs (table 2). Specifically, the impact of larval parasitoids increased nonlinearly with higher gall midge densities (fig. 4).

Table 2: Standardized selection gradients acting on the extended phenotype of egg parasitoids (*Platygaster* sp.)

Selection gradient	**Contrast = Complex - Simple**
β_{Diam}	-0.03 [0.25, -0.3]
β_{Clutch}	0.07 [0.38, -0.26]
β_{Pref}	-0.25 [0.09, -0.64]
$\gamma_{\text{Diam,Diam}}$	-0.05 [0.33, -0.43]
$\gamma_{\text{Clutch,Clutch}}$	-0.21 [0.26, -0.68]
$\gamma_{\text{Pref,Pref}}$	**-0.46 [-0.02, -1.07]**
$\gamma_{\text{Diam,Clutch}}$	0 [0.27, -0.29]
$\gamma_{\text{Diam,Pref}}$	0.25 [0.6, -0.05]
$\gamma_{\text{Clutch,Pref}}$	-0.18 [0.12, -0.52]

Note: Values in brackets represent 95% confidence intervals. Bold values indicate that the 95% CI does not overlap zero. β_{Diam} has been adjusted for bias.

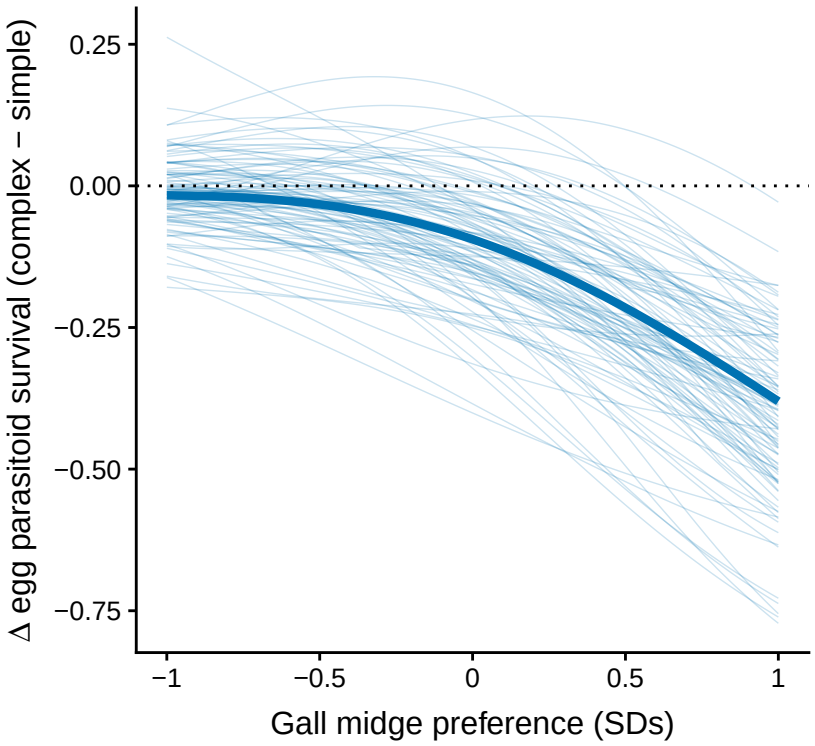

Figure 4: Selection imposed by larval parasitoids on the extended phenotype of egg parasitoids (*Platygaster* sp.). The bold line represents the average difference in the probability of observing the egg parasitoid in complex vs. simple food webs as a function of gall midge oviposition preference. Thin lines represent bootstrapped replicates to show the uncertainty in selection. For clarity, we only display 100 bootstraps even though inferences are based on 1,000 replicates. The decrease in the probability of observing egg parasitoids at high gall-midge densities indicate that larval parasitoids impose nonlinear selection on the extended phenotype of egg parasitoids.

Discussion

We found that simpler food webs constrained phenotypic evolution in gall midges in two key ways. First, more traits contributed to the slope of the adaptive landscape in the simpler food web, suggesting greater constraints on the trajectory of phenotypic evolution. Second, the simple

food-web treatment indirectly increased the number of genetic constraints, which could act to constrain the gall midge's adaptive potential in the face of novel selection pressures. We also found evidence of indirect selection pressures in more complex food webs, suggesting that the adaptive landscape may be more dynamic in complex food webs. Taken together, our study provides experimental evidence from the field that the simplification of ecological communities may constrain the adaptive potential of remaining populations.

All three phenotypic traits we examined experienced directional selection in the simpler food web, indicating that there is an optimal phenotype that maximizes larval survival (i.e. large chamber diameter, small clutch size, and avoidance of conspecifics). In contrast, we did not observe selective constraints on clutch size and oviposition preference in the more complex food web. This suggests that there are many optimal phenotypes (adaptive peaks), or in this case, a flat adaptive landscape where there are no fitness consequences for phenotypic change in these traits. This also implies that as selective constraints dampen in more complex food webs, then the trajectory of evolution becomes more determined by genetic constraints. This is made clear if we inspect the 'Lande equation', $\Delta z = \mathrm{G}\beta$, where G corresponds to the population's G-matrix. As the components of β go to zero, then Δz is more influenced by the G-matrix. Interestingly, this aligns with Schluter (1996)'s hypothesis that phenotypic evolution often follows 'genetic lines of least resistance'. Schluter (1996) found support for this hypothesis from data on natural populations of several vertebrate species, including threespine sticklebacks, a few species of songbirds, and mice from the genus *Peromyscus*. All of these species occupy intermediate trophic levels and are likely embedded in complex food webs, which is consistent with our suggestion that genetic constraints may have a stronger influence in more complex food webs.

We also found evidence for more genetic constraints in simpler food webs due to indirect effects of selection on the population's G-matrix. The ability of a population to adapt to novel selection pressures (evolvability) is largely governed by the structure of its G-matrix (Hansen and Houle, 2008). When selection favors genetic covariance between traits (positive or negative), this results in

less autonomy of evolutionary responses to changing environments. Similarly, decreases in genetic variance constrain potential for the trait itself to evolve. Together, this suggests that simpler food webs may decrease the evolutionary potential of populations by indirectly selecting for decreases in genetic variance in multiple traits and favoring trait integration. Current theory often assumes genetic variances and covariances remain constant over time rather than dynamically changing with the community context (McPeek, 2017; Guimarães et al., 2017). Our empirical results highlight the need to explore the evolutionary consequences of not only direct effects of selection, but indirect effects on genetic constraints that are shaped by the community context.

The generality of our results likely depends on the relative abundance and functional differences between consumers in a community. For example, if consumers do not differ from each other, then we do not expect changes in food-web complexity to modify selective constraints. Also, many consumers may be at too low of abundances to impose selection on their resources. Rank abundance curves (Preston, 1948) and the disproportionate number of weak interactions in diverse communities (Paine, 1992) support this notion. When consumers are abundant though, the effect of food-web complexity will depend on whether different species impose conflicting selection pressures or select for distinct traits. For example, parisitoids and birds impose conflicting selection pressures on the size of galls induced by the fly *Eurosta solidaginis* (Weis and Abrahamson, 1985; Abrahamson and Weis, 1997). Recent studies in this system have shown that decreases in the relative abundance of birds, due to either small patch sizes (Start and Gilbert, 2016) or proximity to urban areas (Start et al., 2018) causes a shift from neutral to directional selection on gall size. On the other hand, different consumers may impose selection on different traits, favoring trait integration and increasing genetic constraints. Examples of this include strong genetic covariances in plant resistance to different insect herbivores (Maddox and Root, 1990; Wise, 2007; Wise and Rausher, 2013), although there are also examples where these covariances are weak (Roche and Fritz, 1997; Barbour et al., 2015), or vary from year-to-year (Johnson and Agrawal, 2007). We suggest that gaining predictive insight to the evolutionary consequences of food-web disassembly requires an understanding of the mechanisms governing the assembly of trophic interactions.

Our results suggest that the simplification of ecological communities may not only directly affect connected species, but also result in indirect evolutionary effects. In our study, this indirect effect arises from egg parasitoids being released from intraguild predation in simpler food webs. This release occurs more on trees with high larval densities, which could intensify selection on gall midge oviposition preference. A growing number of experiments over the past two decades have demonstrated the presence and potential importance of indirect evolutionary effects that emerge in a community context (Juenger and Bergelson, 1998; Stinchcombe and Rausher, 2001; Lankau and Strauss, 2007; Walsh and Reznick, 2008, 2010; terHorst, 2010; Sahli and Conner, 2011; Lau, 2012; terHorst et al., 2015; Schiestl et al., 2018; Start et al., In Press). If indirect evolutionary effects are common (Walsh, 2013), then predicting evolutionary trajectories resulting from the simplification of food webs will require evolutionary studies to explicitly account for the ecological networks that species are embedded in.

There is a growing number of theoretical studies on adaptation to directional environmental change that incorporate species interactions (e.g. de Mazancourt et al., 2008; Johansson, 2008; Norberg et al., 2012; Osmond et al., 2017). This work has given insight to the mechanisms by which pairwise interactions modify the adaptive potential of species. Our study hints at a novel mechanism, whereby food-web complexity flattens the adaptive landscape, thus facilitating future adaptation by allowing genetic and phenotypic variation to persist. This mechanism only emerges once we move beyond pairwise interactions to consider selection on multiple traits in a community context. However, a study on competition has highlighted that we may expect the opposite effect of species diversity in competitive communities (de Mazancourt et al., 2008). This negative effect of species diversity on adaptation occurs because there is a greater chance that the optimal phenotype is already occupied by a competitor in a more diverse community. More work is needed to examine how the distribution of different interaction types affects adaptation to environmental change in species-rich communities.

Our study gives insight to how local extinctions, and concomitant decreases in food-web complexity,

increase evolutionary constraints. This hints at a potential insidious effect of local extinctions that compromises the robustness of remaining populations to future environmental change. Our work also highlights some key challenges for predicting phenotypic evolution in the face of global change. First, the simplification of ecological communities may actually reduce the predictability of phenotypic evolution, because knowledge is required of both selective and genetic constraints, rather than potentially just genetic constraints in more complex systems. Second, many theoretical models of eco-evolutionary dynamics focus on phenotypic change in a single trait, yet our results highlight that the number of traits under selection may change with the community context. Importantly, while the net effects of selection in complex communities may be dampened, we still found that different species/guilds imposed different selection pressures. Knowing these hidden selection pressures is critical for prediction, because the trajectory of evolution will depend on the nature of change in the community context. We expect that a continued integration of adaptive landscapes and ecological networks will enhance our ability to predict the evolutionary consequences of changes in ecological communities.

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