# Food-web complexity simplifies the adaptive landscape of an insect herbivore

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# Abstract

Adaptive landscapes describe the selective constraints acting on phenotypic evolution, but often treat the community context of evolving populations as a "black box". In contrast, ecological networks provide an explicit representation of the community context, yet often ignore the role of natural selection. 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 populations. 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 fewer traits were under selection in complex vs. simple food webs. This occurred because different parasitoid species impose different selection pressures, minimizing relative fitness differences among phenotypes in complex food webs. 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 populations to future environmental change.

# 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, Pfrender, and Jones 2001). More than a metaphor, the adaptive landscape links quantitative genetic and phenotypic variation to evolution by natural selection (Lande 1979; Stevan J. Arnold and Wade 1984a; Stevan J. Arnold and Wade 1984b). 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, Alarcón, and Geber 2006) interactions in driving evolutionary change. Although there is clear evidence that pairwise interactions can shape the adaptive landscape, we also know that most species interact with multiple species in a community context (Darwin 1859). Understanding how the adaptive landscape is shaped by community context represents a major frontier for evolutionary ecologists (McPeek 2017; terHorst et al. 2018). Resolution on this front is urgently needed though, given the rapid impacts of climate change on ecological communities (Scheffers et al. 2016).

Ecological networks, such as a food web describing who eats whom, provide an explicit representation of the community context. These networks describe the interdependency of populations within an ecological community, providing an effective framework for predicting how the strength and organization of ecological interactions (network structure) affect population and community dynamics (Bascompte and Jordano 2014; McCann 2012). Nevertheless, these networks often lack quantitative information on how ecological interactions influence selection on phenotypic traits. Thus, it remains unclear how network structure shapes adaptive landscapes of interacting populations. This represents a major barrier in our current ability to predict how changes in community context will affect phenotypic evolution.

Here, we integrate ecological networks and adaptive landscapes to understand how 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

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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 community context constrains evolutionary change, we must understand how ecological networks shape the adaptive landscape.

To examine the effect of network structure on adaptive landscapes, 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 mortaltiy 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). Food-web complexity may influence the adaptive landscape in a number of ways. For example, if each parasitoid imposes directional selection on a different trait, then more complex food webs may constrain evolution by increasing the number of traits under selection. Alternatively, if parasitoids 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 (among others), we applied modern statistical methods to quantify the effect

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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.

## 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 genotypes) 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,

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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 others work with gall midges (Weis, Price, and Lynch 1983; Heath, Abbot, and Stireman 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, Price, and Lynch 1983; Heath, Abbot, and Stireman 2018). All larvae collected from the same multi-chambered gall were scored with the same clutch size. Third, we measured oviposition (egg laying) preference as the density of larvae observed on a plant in an independent survey. We did this 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 of 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, Cornell, and Hochberg 1997); therefore, we do not expect any prior egg predation to bias our estimates of observed larval densities.

**Quantifying the Adaptive Landscape**

Our analyses 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 ($\alpha_{z_i}$), quadratic ($\alpha_{z_i:z_i}$), and linear interactions ($\alpha_{z_i:z_j}$) between each trait. We also allowed these trait-fitness

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relationships ($\alpha$) 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

this is a mistake since 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 rela-

tionships ($\alpha$) into the scale of relative fitness in order to calculate directional ($\beta_{z_i}$), 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. $\alpha_{z_i}$, $\alpha_{z_i:z_i}$, 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).

**Adaptive landscape**: We took advantage of existing theory that translates estimates of selection gradients to the adaptive landscape (Phillips and Arnold 1989; Arnold 2003). The shape of the adaptive landscape can be described by both its slope and curvature, each of which describe different aspects of constraints on phenotypic evolution. Specifically, the slope corresponds to the column vector of directional selection gradients, which in our study corresponds to:

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

This slope describes the selective constraints acting on the trajectory of phenotypic evolution, which is governed by the 'Lande equation', $\Delta \bar{z} = G\beta$ (Lande 1979). Quantitative predictions of evolutionary trajectories requires knowledge of a population's **G**-matrix; however, we can gain qualitative insight to evolutionary constraints by counting the number of phenotypic traits under directional selection (selective constraints, Arnold 1992).

The curvature (**C**) of the adaptive landscape is a matrix that is determined by directional, quadratic, and correlational selection gradients, such that $C = \gamma - \beta\beta^{\text{T}}$, or more explicitly:

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

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

Note that we ommitted the upper triangle of each matrix for clarity since it is simply the reflection of the lower triangle. This curvature matrix describes the indirect effects of selection on genetic constraints within a generation ($\Delta G = G(\gamma - \beta\beta^{\text{T}})G$). For example, the sign of diagonal terms of

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the curvature matrix dictate 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 (less trait 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 themselves 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 only modelled a linear relationship between chamber diameter and larval survival ($\alpha_{\text{Diam}}$). We detected a positive bias in both food-web treatments (complex $\alpha_{\text{Diam}}$ = 0.36 [0.08, 0.64]; simple $\alpha_{\text{Diam}}$ = 0.34 [-0.03, 0.71]), 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 $\pm\mathbf{1}$ 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, Pfrender, and Jones 2001). We also plot mean larval survival on a natural log scale to accurately reflect the shape of the adaptive landscape (Arnold 2003).

## Results

**Food-web complexity reduces selective constraints**

We found that fewer gall midge traits experienced directional selection in the complex (1 of 3) vs. simple food web (3 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

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selection pressures imposed by each guild of parasitoids (fig. 2C). 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 ($\beta_{\text{Clutch}}$= 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, there was less clear evidence for this relationship in complex food webs (table 1). This was likely a result of the weaker and nonlinear relationship between oviposition preference and fitness consequences imposed by larval parasitoids ($\beta_{\text{Pref}}$= -0.17 [-0.33, -0.03]; $\gamma_{\text{Pref:Pref}}$= 0.18 [-0.02, 0.42]). Food-web complexity did not alter the strong directional selection for larger chamber diameters (table 1; fig. 2).

**Food-web complexity reduces 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. 2). This dampened relationship was partly due to differences in selection imposed by larval parasitoids (contrast $\gamma_{\text{Pref:Pref}}$= -0.21 [-0.5, 0.06]), 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:

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

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

$$\mathbf{C}_{\text{Simple}} = \begin{pmatrix} -0.05 & & \\ 0.02 & -0.01 & \\ 0.04 & -0.01 & -0.03 \end{pmatrix}$$

The structure of these matrices indicate that there are more selective constraints imposed on the gall midge in the simple (6 of 6) vs. complex (1 of 6) food web. Simple 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 complex food web. For genetic covariances, the simple 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 complex food web (fig. 3).

### Selection on the extended phenotype of egg parasitoids

Gall midge preference influenced the probability of observing egg parasitoids in complex vs. simple food webs (contrast $\gamma_{\text{Pref:Pref}}$= 0.47 [-0.01, 1.12]). This indicates that larval parasitoids impose nonlinear selection on this extended phenotype of the egg parasitoid (fig. 4). Larval parasitoids also dampened the linear relationship between probability of observing egg parasitoids and gall midge preference (contrast $\alpha_{\text{Pref}}$= 1.06 [0.08, 2.51] ), but this did not translate into an effect on directional selection (contrast $\beta_{\text{Pref}}$= 0.26 [-0.07, 0.75]). This was likely because there was a tendency (albeit not

statistically signficant) for a higher probability of egg-parasitoid survival in the simple vs. complex food webs (contrast=0.09 [ -0.09, 0.3]). Larval parasitoids did not alter directional, quadratic, or correlational selection on any other extended phenotypes of egg parasitoids (see Supplementary Material).

# Discussion

1. Summary of key results

    (a) Gall midges experience fewer selective and genetic constraints in complex food webs.

2. Natural history and comparison to other studies of natural selection in galling insects.

    (a) **Important Studies for Comparison**: @Abrahamson1997; @Weis1983; @Heath2018; Start, Weis, and Gilbert In Press Am. Nat; @Start2016; @Start2018urbanization; Peter Price's work; Timothy Craig's work; Cheryl Briggs work.

    (b) **Topics to discuss**: Persistent pattern in selection for larger galls; Contrasting effects of egg and larval parasitoids on clutch size; Nonlinear selection at high gall densities is likely reflective of the poorer searching ability of larval vs. egg parasitoids (and thus it saturates more quickly).

3. Selective constraints:

    (a) **Key Result**: Fewer traits under directional selection in complex (1 of 3) vs. simple (3 of 3) food webs. Need to be wary of interpreting no selection on oviposition preference in complex food webs though. Even if there is selection on oviposition preference, this does not change our qualitative result.

    (b) **Implications**: The trajectory of phenotypic evolution is less constrained in complex vs. simple food webs. In other words, there are more potential adaptive peaks in complex food webs.

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4. Indirect effects of selection on genetic constraints:

   (a) **Key Result**: Fewer traits experience a decrease in additive genetic variance and fewer trait combinations experience selection for trait integration.

   (b) **Implications**: The structure of the G-matrix is under less influence of selection in complex vs. simple food webs. Thus the trajectory of phenotypic evolution may be dictated more by genetic rather than selective constraints. This is in line with empirical evidence (from complex natural systems) that evolution tends to proceed along genetic lines of least resistance [@Schluter1996].

5. Indirect evolutionary effects:

   (a) **Key Result**: Evidence of nonlinear selection on the extended phenotype of egg parasitoids.

   (b) **Implications**: Hints at a dynamic adaptive landscape in complex food webs.

6. Caveats:

   (a) **Key Caveat**: Experimental artifact of lower larval survival in complex food-web treatment.

   (b) **Implications**: Artificially increased the magnitude of selection in complex food webs. Note however, that this does not qualitatively change any of our results. In fact, we are actually underestimating the constraints imposed by our simple food-web treatment. This is because the egg parasitoids would be released from their intraguild predator, and thus could increase in abundance over future generations and impose stronger selection on gall midges.

7. Conclusions

## Acknowledgements

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# Tables

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

| Selection gradient | Complex | Simple | Contrast |
|---|---|---|---|
| $\beta_{\text{Diam}}$ | **0.34 [ 0.23, 0.49]** | **0.22 [ 0.13, 0.31]** | -0.12 [ -0.26, 0.01] |
| $\beta_{\text{Clutch}}$ | 0.06 [ -0.05, 0.18] | **-0.09 [ -0.17, -0.01]** | **-0.15 [ -0.29, -0.02]** |
| $\beta_{\text{Pref}}$ | -0.12 [ -0.29, 0.04] | **-0.16 [ -0.26, -0.07]** | -0.04 [ -0.23, 0.14] |
| $\gamma_{\text{Diam,Diam}}$ | 0.13 [ -0.04, 0.32] | 0.1 [ -0.02, 0.23] | -0.04 [ -0.26, 0.19] |
| $\gamma_{\text{Clutch,Clutch}}$ | -0.05 [ -0.28, 0.14] | -0.11 [ -0.27, 0.03] | -0.06 [ -0.32, 0.2] |
| $\gamma_{\text{Pref,Pref}}$ | **0.34 [ 0.08, 0.66]** | 0.01 [ -0.15, 0.17] | **-0.33 [ -0.68, -0.03]** |
| $\gamma_{\text{Diam,Clutch}}$ | -0.04 [ -0.17, 0.09] | -0.07 [ -0.16, 0.01] | -0.03 [ -0.18, 0.12] |
| $\gamma_{\text{Diam,Pref}}$ | -0.13 [ -0.28, 0.01] | -0.02 [ -0.09, 0.07] | 0.11 [ -0.04, 0.28] |
| $\gamma_{\text{Clutch,Pref}}$ | 0.03 [ -0.11, 0.18] | 0 [ -0.07, 0.08] | -0.03 [ -0.19, 0.13] |

Note: Values in brackets represent 95% confidence intervals. Bold values indicate that the 95% CI does not overlap zero.

$\beta_{\text{Diam}}$ has been adjusted for bias.

Table 2: Relationship between absolute fitness (larval survival) and phenotypic traits of gall midges in complex vs. simple food webs.

| Coefficient | Complex | Simple | Contrast |
|---|---|---|---|
| $\alpha_{\text{Diam}}$ | **1.15 [ 0.75, 1.63]** | **1.18 [ 0.71, 1.67]** | 0.03 [ -0.5, 0.57] |
| $\alpha_{\text{Clutch}}$ | 0.21 [ -0.15, 0.6] | **-0.47 [ -0.88, -0.07]** | **-0.68 [ -1.25, -0.14]** |
| $\alpha_{\text{Pref}}$ | -0.4 [ -0.98, 0.14] | **-0.85 [ -1.36, -0.39]** | -0.45 [ -1.17, 0.24] |
| $\alpha_{\text{Diam,Diam}}$ | 0.22 [ -0.07, 0.53] | 0.26 [ -0.05, 0.62] | 0.04 [ -0.43, 0.51] |
| $\alpha_{\text{Clutch,Clutch}}$ | -0.09 [ -0.46, 0.24] | -0.3 [ -0.71, 0.08] | -0.21 [ -0.76, 0.3] |
| $\alpha_{\text{Pref,Pref}}$ | **0.57 [ 0.13, 1.09]** | 0.02 [ -0.39, 0.44] | -0.54 [ -1.25, 0.04] |
| $\alpha_{\text{Diam,Clutch}}$ | -0.14 [ -0.55, 0.29] | -0.36 [ -0.83, 0.03] | -0.22 [ -0.82, 0.36] |
| $\alpha_{\text{Diam,Pref}}$ | -0.43 [ -0.94, 0.04] | -0.09 [ -0.49, 0.35] | 0.34 [ -0.28, 0.99] |
| $\alpha_{\text{Clutch,Pref}}$ | 0.09 [ -0.36, 0.59] | 0.01 [ -0.37, 0.4] | -0.08 [ -0.69, 0.53] |

Note: Values in brackets represent 95% confidence intervals. Bold values indicate that the 95% CI does not overlap zero.

$\alpha_{\text{Diam}}$ has been adjusted for bias.
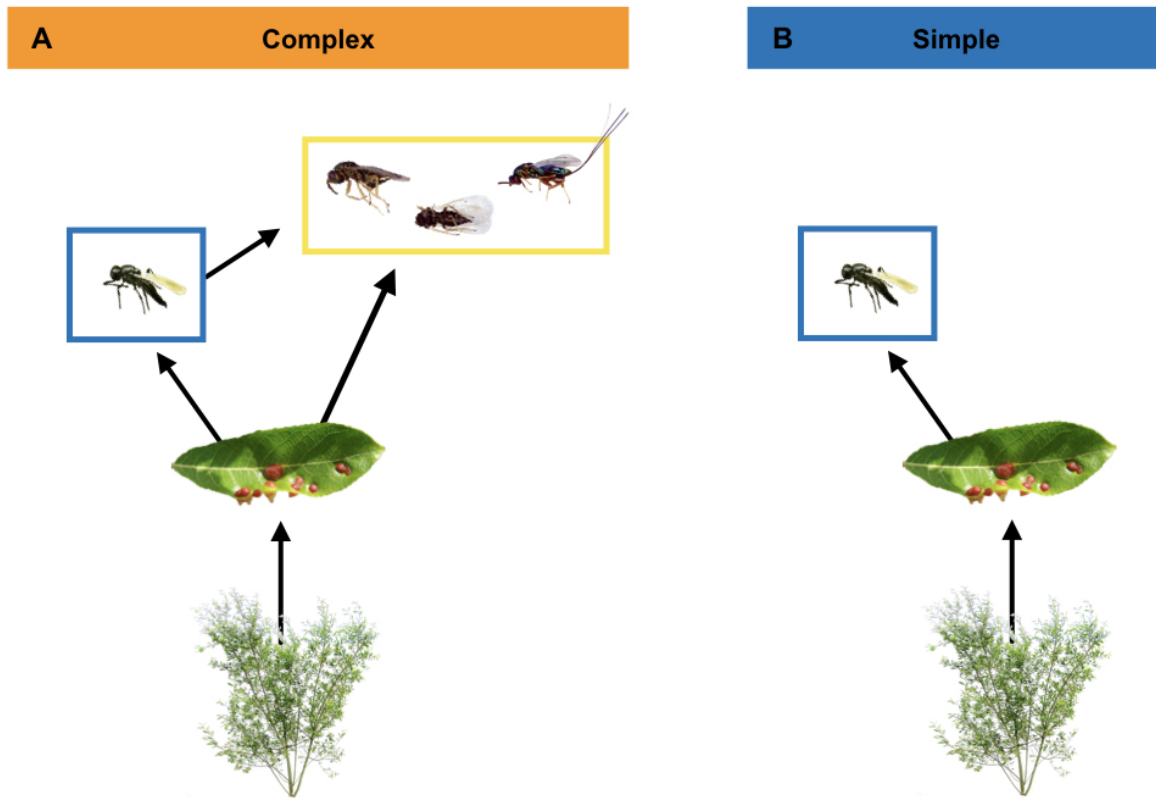
## Figure Legends

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

**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). Solid lines represent selection experienced in complex (orange) and simple (blue) food webs. Transparent 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. Solid yellow lines represent selection imposed by larval parasitoids in the complex food web. Note that mean larval survival is plotted on a natural log scale to accurately reflect the adaptive landscape.

**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). Selection favors trait integration for each combination of traits in simple food webs, whereas there is no strong evidence for trait integration in complex food webs. Note that mean larval survival is plotted on a natural log scale to accurately reflect the adaptive landscape.

**Figure 4**: Selection imposed by larval parasitoids on the extended phenotype of egg parasitoids (*Platygaster* sp.). The solid line represents the average difference in the probability of observing the

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egg parasitoid in complex vs. simple food webs as a function of gall midge oviposition preference. Transparent 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.

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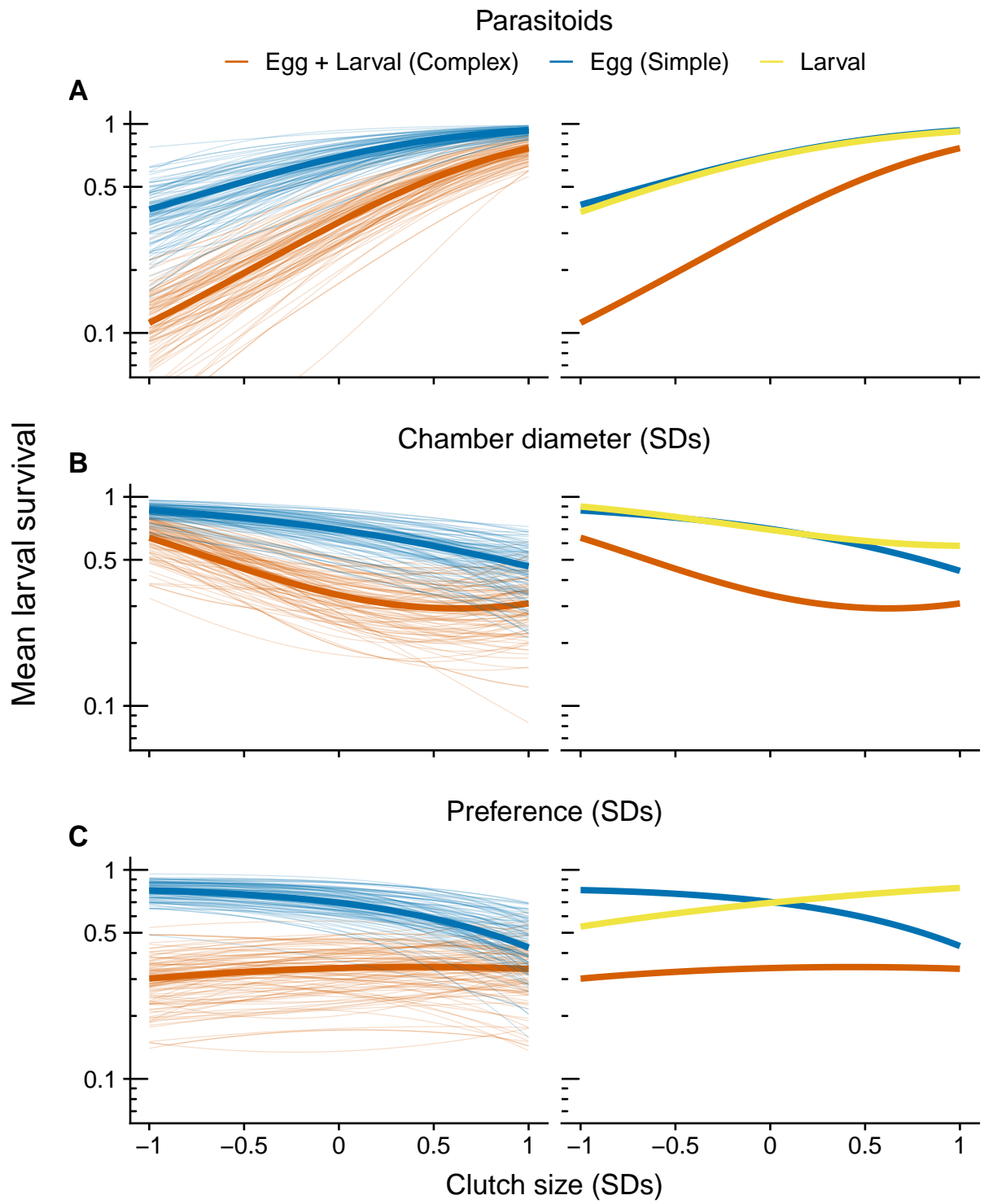
Parasitoids

Egg + Larval (Complex) — Egg (Simple) — Larval

**A**

Chamber diameter (SDs)

**B**

Preference (SDs)

**C**

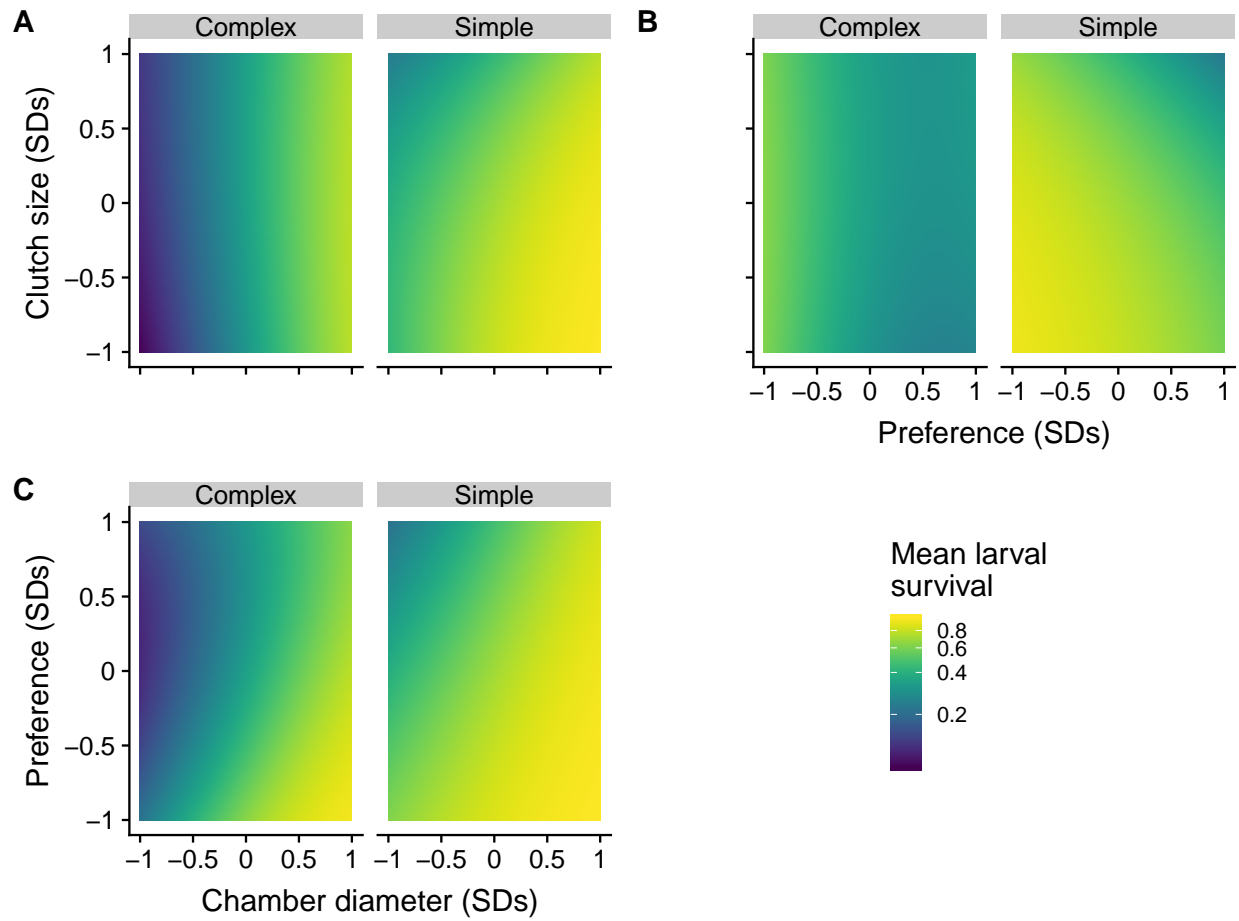Mean larval survival

Clutch size (SDs)

Figure 2:

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). Selection favors trait integration for each combination of traits in simple food webs, whereas there is no strong evidence for trait integration in complex food webs. Note that mean larval survival is plotted on a natural log scale to accurately reflect the adaptive landscape.
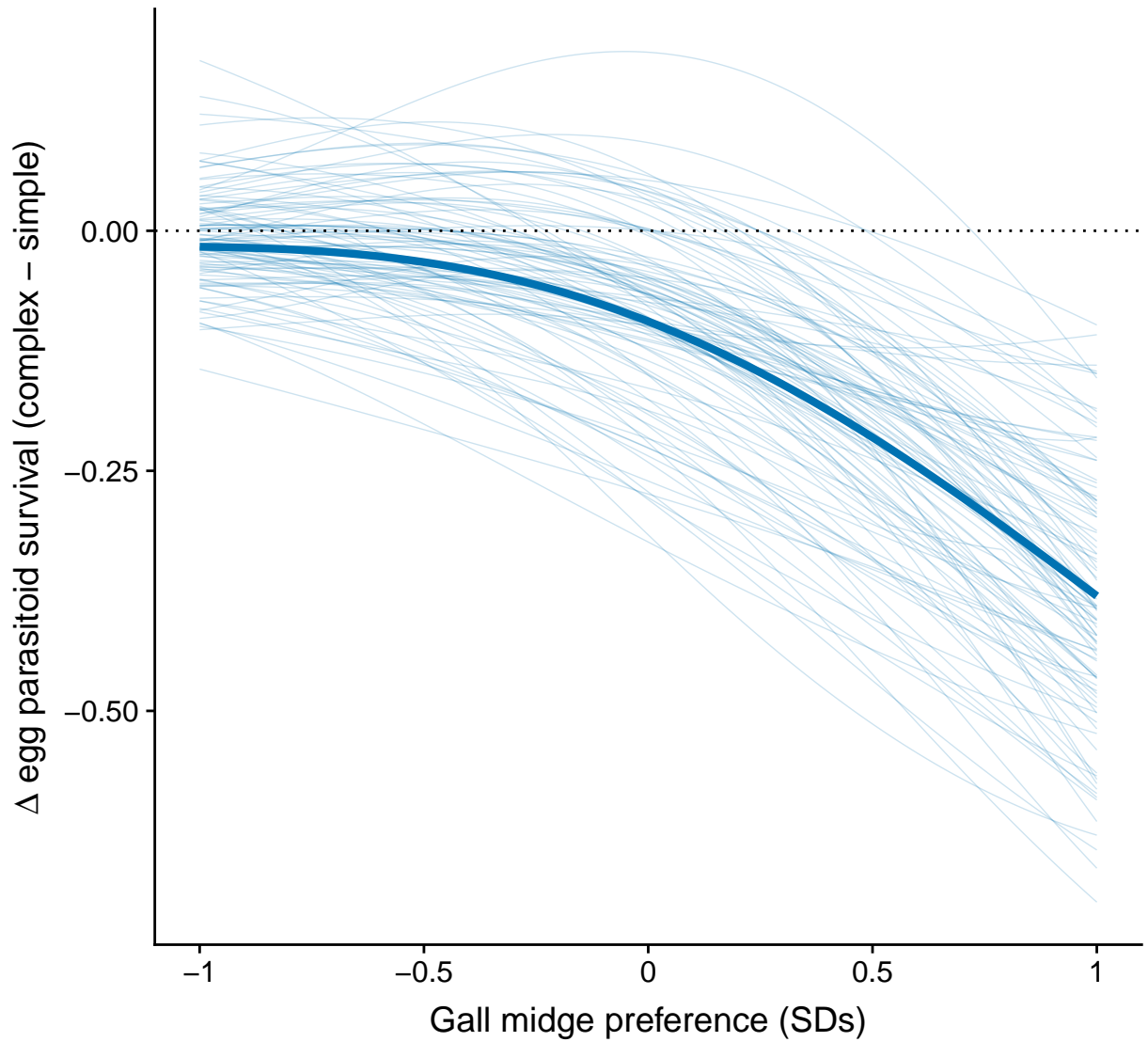
Figure 4: Selection imposed by larval parasitoids on the extended phenotype of egg parasitoids (*Platygaster* sp.). The solid line represents the average difference in the probability of observing the egg parasitoid in complex vs. simple food webs as a function of gall midge oviposition preference. Transparent 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.