

Phenotypic evolution is more constrained in simpler food webs

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Manuscript elements: Figure 1, figure 2, figure 3, figure 4. All figures should be printed in color.

Keywords: fitness landscape, host-parasitoid, natural selection, G-matrix, community context, ecological networks.

Manuscript type: Article.

Prepared using an *Am. Nat.* inspired L^AT_EX template for Rmarkdown.

Abstract

Global change is simplifying the structure of ecological networks; however, we are currently in a
3 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 treat the community context of evolving
6 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)
9 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
12 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
15 may constrain the adaptive potential of remaining populations to future environmental change.

Introduction

The adaptive landscape provides a powerful framework for understanding how natural selection
18 has shaped the evolution of biodiversity —from genes to phenotypes to species (???). More
than a metaphor, the adaptive landscape links quantitative genetic and phenotypic variation to
evolution by natural selection (???). Ecological interactions often play a key role in shaping adaptive
21 landscapes, as evidenced by the role of antagonistic (??) and mutualistic (?) interactions in driving
evolutionary change. Although there is clear evidence that pairwise interactions can shape the
adaptive landscape, we do not know how the adaptive landscape is shaped by the community
24 context (??). Resolution on how the community context shapes phenotypic evolution is urgently
needed though, given the rapid impacts of climate change on ecological communities (?).

Ecological networks, such as food webs describing who-eats-whom in ecological communities,
27 provide an explicit representation of the community context (??). Here, we integrate ecological
networks and adaptive landscapes to understand how community context constrains evolutionary
change (?). Different aspects of evolutionary constraints can be inferred by quantifying the slope
30 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 (??). Evolutionary trajectories become more constrained with an increase in the number of
33 traits under selection, as this diminishes the number of optimal solutions (?). The curvature of the
adaptive landscape can also constrain evolution through its indirect effect on genetic constraints
(??). Genetic constraints are largely governed by a population's **G**-matrix —the additive genetic
36 variances and covariances between traits (?). 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 (?). Genetic constraints may also increase with the number of trait
39 combinations under correlational selection, as this type of selection decreases the evolutionary
independence of traits (?). If we want to predict how community context constrains evolutionary
change, we must understand how ecological networks shape the adaptive landscape.

42 Global change is simplifying the structure of ecological communities, which may influence evolu-
tionary 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
45 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
48 (among others), 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
51 when they feed on the developing leaves of willow trees (*Salix* sp., ?). These galls protect larva
from attack by generalist predators (e.g. ants, spiders), but they suffer high mortality from egg
and larval parasitoids (?). We manipulated food-web complexity by either excluding the guild of
54 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
57 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

60 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)
63 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

66 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
69 in ?.

Manipulating Food-web Complexity

We setup our food-web manipulation across 128 plants soon after galls began developing on
72 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 (?). For the complex
food-web treatment (eight replicates per genotype), we used flagging tape to mark 14 galled leaves
75 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
78 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
81 in Cecidomyiid midges: ?). 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
84 (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
87 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
90 111 plants.

Measuring Phenotypic Traits

We collected data on three different traits that we expected to influence larval survival based on our previous work (?) and others work with gall midges (??). 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 (?). Second, we measured clutch size by counting the number of chambers in each gall (??). 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 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 (?); however, caution must be taken in inferring ‘preference’ as larval densities can be influenced by processes other than preference (?). 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 (?); 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
117 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 (?). To approximate this assumption, we log-transformed
120 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.

123 **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}),
126 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 (?).
129 Other approaches have been advocated for approximating selection surfaces (?); however, our
approach enables us to calculate selection gradients, and thus is more appropriate for approximating
the adaptive landscape (?). To account for the nonindependence of clutch size (measured at gall
132 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
135 analyses of natural selection, we think that modelling random effects can mitigate biased estimates
of selection due to environmental covariances between traits and fitness (?). Since our end goal was
to characterize the relationship between mean trait values and mean fitness (adaptive landscape),
138 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
141 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 ? to translate trait-fitness relationships (α) into the

144 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 $\alpha_{z_i:z_j}$).
 147 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
 150 gradients to put them on the same scale as estimates of directional and correlational selection (?).

Evolutionary constraints: We quantified the effects of food-web complexity on evolutionary con-
 straints by inspecting the slope and curvature of the adaptive landscape. The number of selective
 153 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
 156 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
 159 landscape, 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 omitted 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 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 (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. $1 > \text{mean survival} > 0$). 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 (α_{Diam}). We detected a positive bias in both food-web treatments (complex $\alpha_{\text{Diam}} = 0.36 [0.05, 0.67]$; simple $\alpha_{\text{Diam}} = 0.42 [0.01, 0.82]$), indicating that unadjusted relationships would overestimate the strength of selection on chamber diameter. To account for this bias, we subtracted 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 (?). 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 (?). We also plot mean larval survival on a natural log scale to accurately reflect the shape of the adaptive landscape (?).

Results

Food-web complexity reduces selective constraints

We found that more gall midge traits experienced directional selection in the simple (3 of 3) vs. complex food webs (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
 207 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 (fig. 2C). Specifically, when we subset our
 data to focus on differences between parasitoid guilds, we found that larval parasitoids actually
 210 impose directional selection for larger clutch sizes ($\beta_{\text{Clutch}} = 0.13 [0.03, 0.23]$). 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
 213 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
 216 experienced positive directional selection in both food-web treatments, but selection was 65%
 higher in complex food webs (table 1; fig. 2). This was not due to any difference between egg and
 larval parasitoids (fig. 2), but was due to the lower average survival in complex food webs (contrast
 219 $-0.26 [-0.12, -0.42]$).

Food-web complexity reduces genetic constraints

The curvature of the adaptive landscape indirectly affects genetic constraints and is influenced by
 222 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).
 225 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
 228 partly due to a trend for nonlinear selection imposed by larval parasitoids ($\gamma_{\text{Pref:Pref}} = 0.18 [-0.01,$
 $0.42]$), 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 ($\gamma - \beta\beta^T$) in each food-web treatment. We found that the curvatures of the adaptive landscape exhibited the following structures:

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

$$\mathbf{C}_{\text{Complex}} = \begin{pmatrix} -0.12 & & \\ 0 & 0 & \\ 0 & 0 & 0.33 \end{pmatrix}$$

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

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).

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 $\alpha_{\text{Pref}} = 1$ [0.03, 2.22]). Specifically, larval parasitoids decreased the probability of observing egg parasitoids at high gall midge densities (fig. 4). Note however that this trait-fitness relationship did not translate into a significant difference in selection (contrast $\beta_{\text{Pref}} = 0.24$ [-0.09, 0.62]). This was likely because there was a tendency (albeit not statistically significant) for a lower probability of egg-parasitoid survival in complex vs. simple food webs (contrast=0.1 [-0.08, 0.31]), which masks this trait-fitness relationship. We also observed two other interesting trends, although neither significantly differed between treatments. Specifically, there was a tendency for the impact of larval parasitoids to increase nonlinearly at higher gall midge densities (fig. 4; complex $\alpha_{\text{Pref:Pref}} = -0.49$ [-1.08, -0.1]; but contrast $\alpha_{\text{Pref:Pref}} = 0.46$ [-0.03, 1.1]). There was also a tendency for egg parasitoids to obtain a refuge in large gall chambers at high gall midge densities (complex $\alpha_{\text{Pref:Diam}} = 0.25$ [0.02, 0.5]; but contrast $\alpha_{\text{Pref:Diam}} = -0.25$ [-0.57, 0.04]).

Discussion

1. Summary of key results

(a) Gall midges experience more selective and constraints in simple 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:** More traits under directional selection in simple (3 of 3) vs. complex (1 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.

4. Indirect effects of selection on genetic constraints:

(a) **Key Result:** More traits experience a decrease in additive genetic variance and more trait combinations experience selection for trait integration in simple food webs.

(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 selection on the extended phenotype of egg parasitoids.

(b) **Implications:** Hints at a dynamic adaptive landscape and potential indirect evolutionary effects 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

References

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, 0.47]	0.21 [0.13, 0.3]	-0.13 [-0.26, 0.01]
β_{Clutch}	0.06 [-0.04, 0.17]	-0.09 [-0.18, -0.02]	-0.15 [-0.29, -0.02]
β_{Pref}	-0.12 [-0.29, 0.05]	-0.16 [-0.25, -0.07]	-0.04 [-0.22, 0.14]
$\gamma_{\text{Diam,Diam}}$	0.14 [-0.06, 0.32]	0.1 [-0.03, 0.25]	-0.04 [-0.27, 0.2]
$\gamma_{\text{Clutch,Clutch}}$	-0.05 [-0.25, 0.16]	-0.12 [-0.27, 0.03]	-0.06 [-0.33, 0.19]
$\gamma_{\text{Pref,Pref}}$	0.33 [0.06, 0.64]	0.01 [-0.14, 0.17]	-0.32 [-0.66, 0]
$\gamma_{\text{Diam,Clutch}}$	-0.05 [-0.17, 0.08]	-0.07 [-0.15, 0.02]	-0.02 [-0.16, 0.13]
$\gamma_{\text{Diam,Pref}}$	-0.13 [-0.28, 0]	-0.02 [-0.09, 0.06]	0.12 [-0.05, 0.29]
$\gamma_{\text{Clutch,Pref}}$	0.03 [-0.11, 0.18]	0 [-0.07, 0.07]	-0.03 [-0.19, 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.

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

Coefficient	Complex	Simple	Contrast
α_{Diam}	1.14 [0.73, 1.57]	1.1 [0.68, 1.58]	-0.04 [-0.57, 0.51]
α_{Clutch}	0.21 [-0.13, 0.57]	-0.47 [-0.94, -0.09]	-0.68 [-1.27, -0.13]
α_{Pref}	-0.41 [-0.97, 0.15]	-0.84 [-1.35, -0.36]	-0.43 [-1.16, 0.25]
$\alpha_{\text{Diam,Diam}}$	0.23 [-0.1, 0.54]	0.27 [-0.07, 0.65]	0.04 [-0.43, 0.52]
$\alpha_{\text{Clutch,Clutch}}$	-0.09 [-0.42, 0.26]	-0.31 [-0.71, 0.08]	-0.22 [-0.77, 0.31]
$\alpha_{\text{Pref,Pref}}$	0.56 [0.1, 1.07]	0.03 [-0.38, 0.46]	-0.52 [-1.18, 0.09]
$\alpha_{\text{Diam,Clutch}}$	-0.16 [-0.57, 0.25]	-0.35 [-0.78, 0.08]	-0.19 [-0.78, 0.44]
$\alpha_{\text{Diam,Pref}}$	-0.44 [-0.95, 0.01]	-0.09 [-0.5, 0.3]	0.36 [-0.27, 0.97]
$\alpha_{\text{Clutch,Pref}}$	0.1 [-0.37, 0.59]	0 [-0.37, 0.38]	-0.1 [-0.69, 0.49]

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

α_{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. Note that mean larval survival is plotted on a natural log scale to reflect the mathematical definition of 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). Note the flat landscape in mean larval survival for clutch size and oviposition preference in more complex food webs, whereas all three traits shape the adaptive landscape in simpler food webs. This indicates that selection favors trait integration for each combination of traits in simpler food webs, whereas there is no strong evidence for trait integration in 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.

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 indicates the increasing impact of larval parasitoids.

Appendix

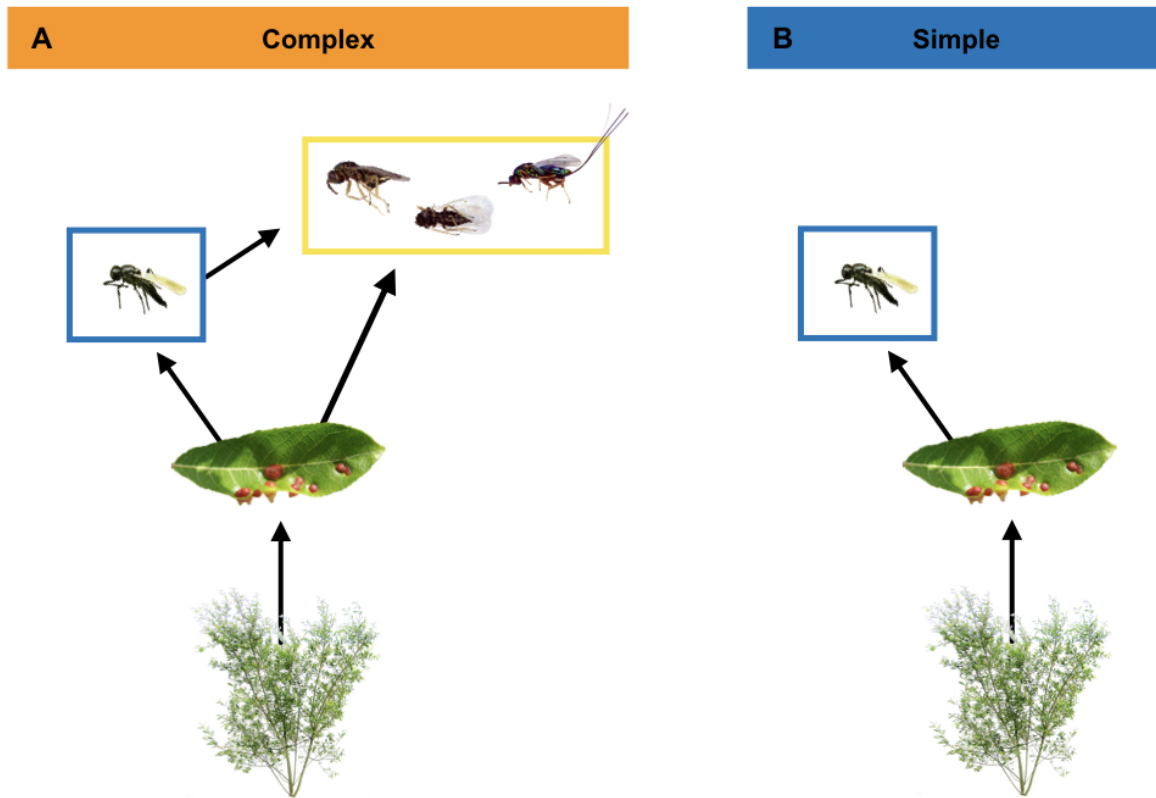


Figure 1: Experimental manipulation of food-web complexity associated with a leaf-galling midge (*Iteomyia salicisverruca*) feeding on the willow *Salix hookeriana*. In the complex food-web treatment (A), we allowed the full suite of egg (blue rectangle) and larval (yellow rectangle) parasitoids to impose 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).

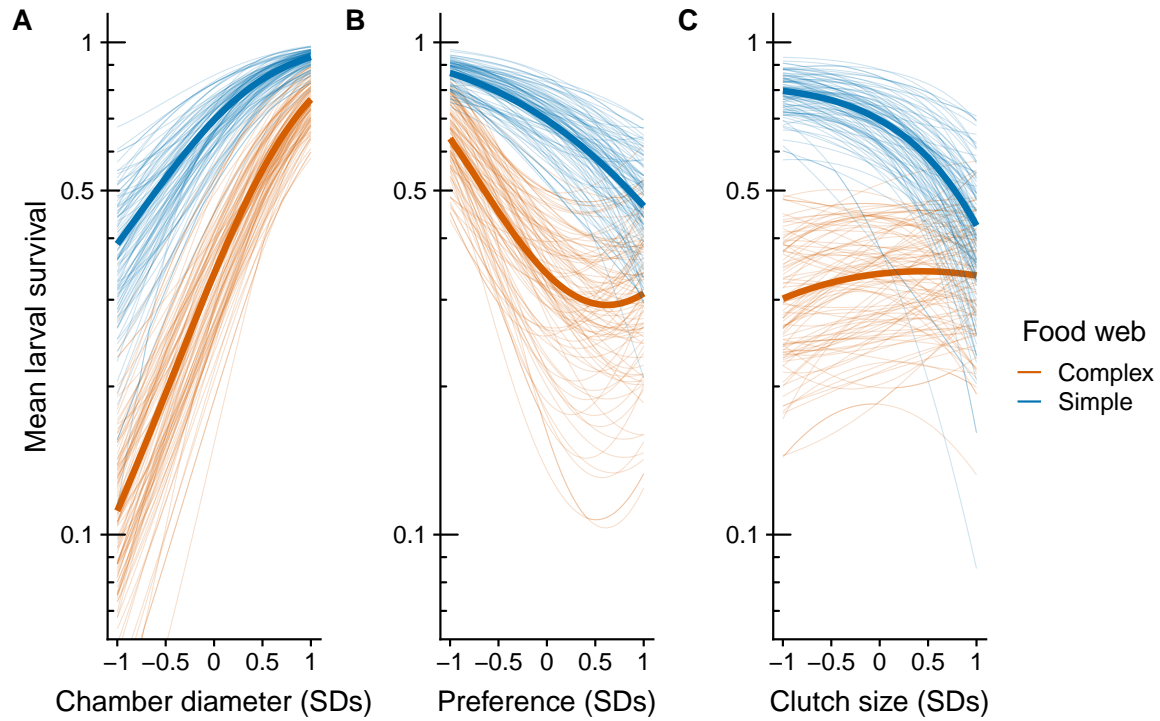


Figure 2: Adaptive landscape of gall midge phenotypes in complex vs. simple food webs. Each panel corresponds to a different phenotypic trait: chamber diameter (A); oviposition preference (B); and clutch size (C). Solid lines represent selection experienced in complex (orange) and simple (blue) food 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. Note that mean larval survival is plotted on a natural log scale to reflect the mathematical definition of the adaptive landscape.

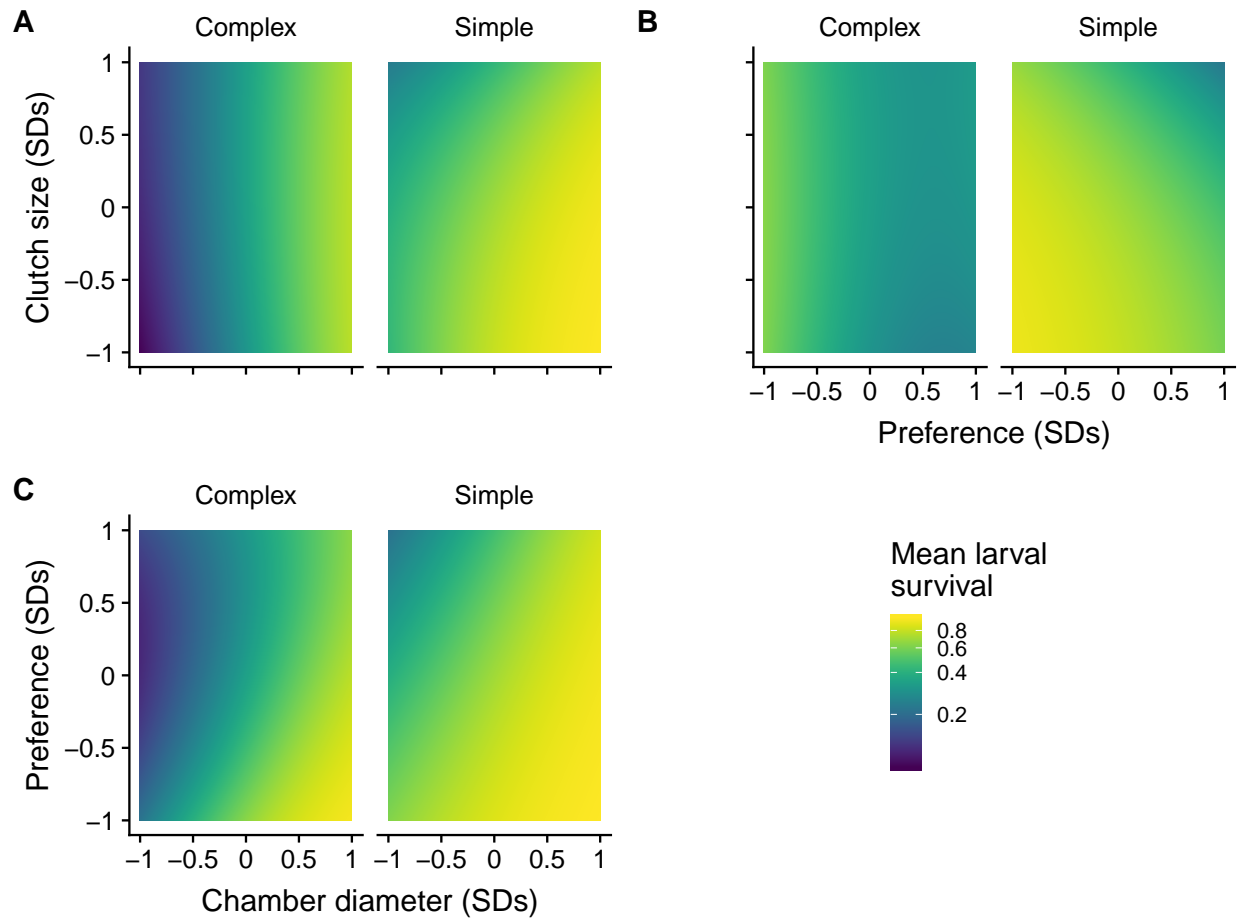


Figure 3: Two dimensional view of adaptive landscapes of gall midge phenotypes in complex vs. simple food webs. Each panel corresponds to a different combination of phenotypic traits: clutch size and chamber diameter (A); clutch size and oviposition preference (B); oviposition preference and chamber 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.

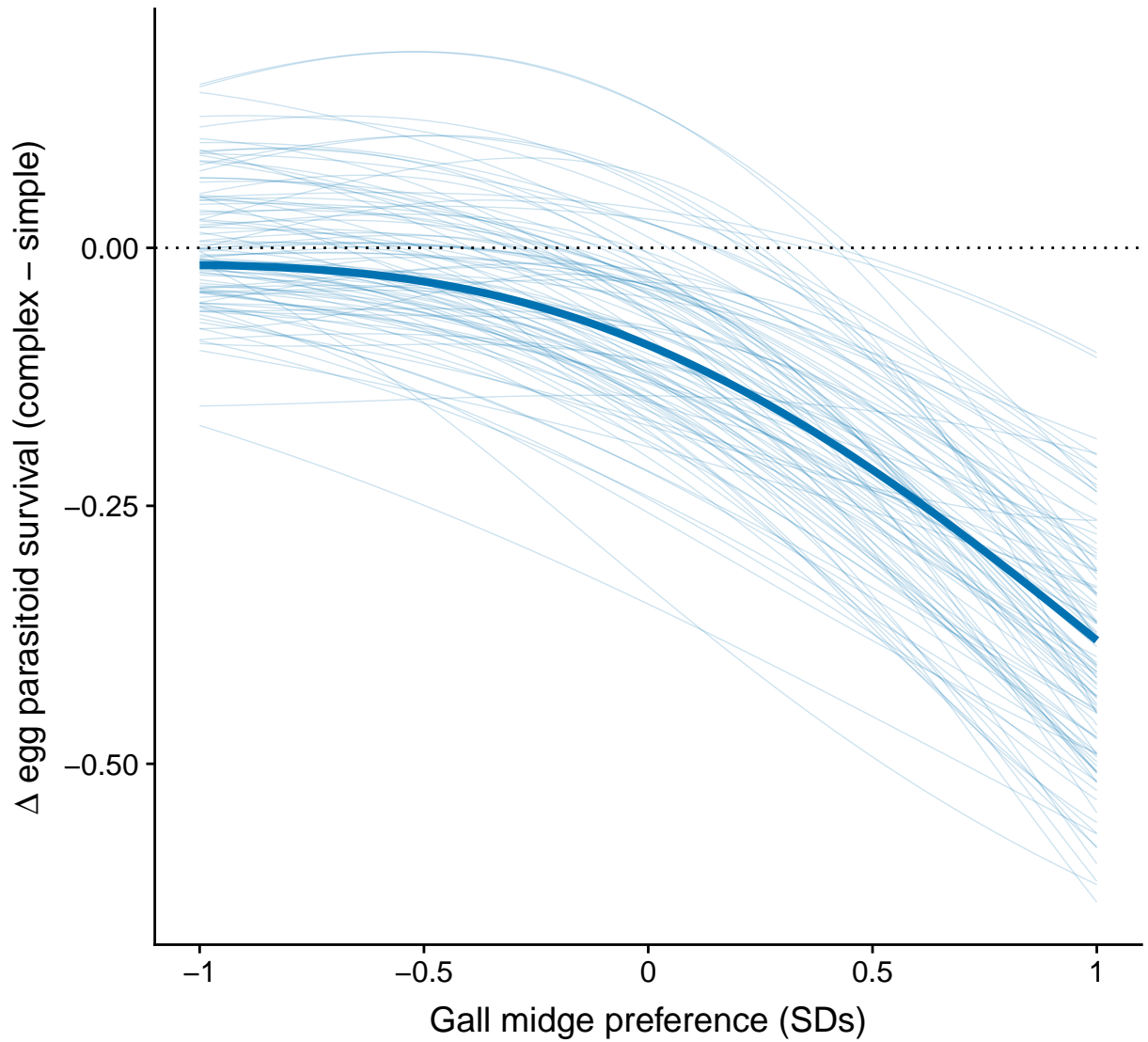


Figure 4: Selection imposed by larval parasitoids on the extended phenotype of egg parasitoids (*Platygaster* sp.). The solid line represents the average difference in the probability of observing the egg parasitoid in complex vs. simple food webs as a function of gall midge oviposition preference. Transparent 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.