

Food-web complexity flattens the fitness landscape of an insect herbivore

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Abstract

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*There is a much more insidious kind of extinction: the extinction of ecological interactions. (Janzen, D.H. 1974. *The deflowering of Central America. Natural History.* 83:48–53).*

Introduction

Biological diversity – from genes, to phenotypes, to species – has and continues to be shaped by the interplay between ecological and evolutionary processes. Much of this biological diversity has been molded by natural selection arising from species interactions, such as resource competition (Schluter (2000)), mutualisms (Jordano (1987)), and predation (Abrams (2000)). While there is clear evidence that pairwise interactions can drive evolution, we also know that most species interact with multiple species in an ecological community. Understanding how evolutionary dynamics unfold in a community context is challenging and eminently theoretical (Mcpeak (2017), Mazancourt, Johnson, and Barracough (2008), Guimarães et al. (2017), Nuismer, Jordano, and Bascompte (2013)). Given the rapid loss of species diversity we are experiencing throughout the world (cite), we are in urgent need of work that makes and tests predictions for how the loss of species will affect the evolutionary process in natural communities.

Predicting the evolutionary consequences of species loss first requires an understanding of the concomitant change in the species-interaction networks. Knowing the interaction network is crucial because the loss of biodiversity, in and of itself, will not alter evolution – it is the associated loss of ecological interactions that will affect evolutionary change (Janzen (1974)). For a network of directly connected species, we would expect that the loss of species to result in a loss of network complexity. Network complexity is a property that describes the diversity of interactions in an ecological community (Banasek-Richter et al. (2009)). Thus, all else equal, species loss will decrease the diversity of interactions, resulting in a more simple network.

Predicting how a change in network complexity will alter evolution also requires an understanding of the relationship between network structure and the adaptive landscape. The adaptive landscape (i.e. fitness landscape or selective surface) describes the relationship between the average trait value of a population and its average fitness (cite Arnold). For a trophic network, such as a food web, changes in network complexity can shape the adaptive landscape of constituent species in at least two ways. First, if a more diverse community of consumers is more efficient at suppressing resource densities (Ives, Cardinale, and Snyder (2005)), then this will result in lower mean fitness of the resource population. A reduction in mean fitness, all else equal, will intensify natural selection (Hunter et al. (2018)) and thus could speed up the rate of evolutionary change. On the other hand, if consumers are functionally distinct, then more diverse communities can dampen the strength of selection. This is

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because each consumer has a different functional relationship with resource traits. In addition, there is a reduced probability in interacting with a specific consumer species in a more diverse community. Thus, a more diverse consumer community may impose more diffuse selection across the adaptive landscape.

Here, we provide a quantitative test of how the loss of species diversity – and concomittant loss of network complexity – shapes the adaptive landscape of a constituent species in a natural community. We conducted a field experiment that manipulated the diversity of insect parasitoids that were able to impose selection on the insect herbivore, *Iteomyia salicisverruca*. The larva of this herbivore species induces tooth-shaped galls when they feed on the developing leaves of willow trees (*Salix* sp., Russo (2006)). Prior work with this study system has shown that there is directional selection for larger galls, likely because larger galls provide a refuge from parasitoid attack (Barbour et al. (2016)). However, there is also evidence that each parasitoid species imposes differential selection on gall traits (Barbour et al. (2016)). Taken together, our aim is to provide evidence for how the simplification of natural communities affects the adaptive potential of constituent species.

Materials & Methods

Study Site

We conducted our study within a four-year old common garden of coastal willow (*Salix hookeriana*) located at Humboldt Bay National Wildlife Refuge (HBNWR) ($40^{\circ}40'53''N$, $124^{\circ}12'4''W$) 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 in our garden 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).

Food-Web Manipulation

We setup our food-web manipulation across 128 plants soon after galls began developing on *S. hookeriana* in early June of 2013. These 128 plants came from eight different plant genotypes, spanning the range of trait variation observed in this willow population (Barbour et al. (2015)). On treatment plants (8 replicates per genotype), we enclosed 14 galled leaves with organza bags (MANUFACTORER DETAILS) to exclude three parasitoid species that attack during larva development (hereafter larval parasitoids). This treatment did not exclude the egg parasitoid *Platygaster* sp. which attacks prior to gall initiation (note that in Cecidomyiid midges, larva initiate gall development CITE). On control plants (8 replicates per genotype), we used flagging tape to mark 14 galled leaves per plant, allowing the full suite of parasitoids to attack *Iteomyia*. Marking galls with flagging tape ensured that we compared control and treatment galls with similar phenology when we collected galls later in the season. Our food-web manipulation altered the average number of trophic interactions that *Iteomyia* was exposed to from BLANK on control plants to BLANK on treatment plants. Thus, we refer to galls on control plants as being exposed to a ‘complex’ food web, whereas galls on treatment plants were exposed to a ‘simple’ food web. 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 larva survived to pupation (our measure of fitness) or were parasitized.

Measuring Gall Traits

We collected data on three different traits that we anticipated would experience selection based on our previous work (Barbour et al. (2016)) and others work with Cecidomyiid midges (Weis, Price, and Lynch (1983), J. J. 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 a larger gall diameter provides a refuge for larva from parasitoid attack (Barbour et al. (2016)). Second, we measured the clutch size of adult female midges by counting the number of chambers in each

gall (Weis, Price, and Lynch (1983)). All larva collected from the same multi-chambered gall were scored with the same clutch size. Third, we measured female preference for oviposition (egg-laying) sites as the density of larva observed on a plant. The measurement of larval densities on plants in the field is a commonly used index for measuring oviposition preference (Gripenberg et al. (2010)), although caution must be taken in inferring ‘preference’ (Singer (1986)). This is because larval densities can be influenced by processes other than preference. For example, if an ovipositing female is not exposed to the full spectrum of plant types (in this case genotypes), then it is difficult to infer whether patterns of larval densities are actually due to preference. Also, observed larval densities could be influenced by egg predation.

While we recognize these limitations, a couple of aspects of our study system likely alleviate these limitations. For example, since our data comes from a randomized placement of willow genotypes in a common garden, there is no consistent bias in which willow genotypes that females are exposed to while searching for oviposition sites. Although we cannot control for egg predation, this source of mortality appears to play comparatively minor role in determining the mortality of galling insects (Hawkins, Cornell, and Hochberg (1997)). To quantify female preference (gall density), we randomly sampled five branches per tree and summed the number of individual gall chambers observed. We converted these counts to a measure of gall density per 100 shoots by counting the number of shoots on the last branch we sampled. All larva collected from the same plant were scored with the same female preference.

Statistical Analyses

To characterize the shape of the fitness landscape, we quantified selection gradients acting on each trait in simple vs. complex food webs. We did this by fitting separate statistical models to data from each food-web treatment. We used generalized linear mixed models (GLMMs, Bolker et al. (2009)) with larval survival (0 or 1) as our response variable and measure of fitness. We specified linear and quadratic terms for each gall trait as well as linear interaction terms between each gall trait as fixed effects in the statistical models. To account for the correlated structure of clutch size (gall level) and female preference (plant level) as well as any other independent effects of willow genotype on larval survival, we specified gall ID nested within plant ID nested within plant genotype as random intercepts in our statistical models. Since we were interested in characterizing the fitness landscape – the relationship between mean trait values and population mean fitness – we assumed the mean value of our random effects (i.e. setting them to zero) to estimate selection gradients. We then used the method of Frederic J Janzen and Hal S Stearn (1998) to calculate directional (β_{z_i}), quadratic (γ_{z_i}), and correlational (γ_{z_i, z_j}) selection gradients and used parametric bootstrapping (1000 replicates) to calculate their 95% confidence intervals (Bolker et al. (2009)). To test whether selection gradients differed between treatments, we used our bootstrapped estimates to calculate the probability that selection gradients in the simple food web were larger/smaller than in the complex food web (i.e. the p-value). All analyses and visualizations were conducted in R (R Core Team (2018)).

```
##          Phenotype Food_Web Model Type Estimate lower_2.5 upper_97.5
## 1      Gall diameter    Complex GLMM Beta    0.555    0.432    0.642
## 2      Clutch size     Complex GLMM Beta    0.036   -0.075    0.177
## 3 Female preference    Complex GLMM Beta    0.003   -0.228    0.148
## 4      Gall size       Simple  GLMM Beta    0.289    0.220    0.357
## 5      Clutch size     Simple  GLMM Beta   -0.102   -0.190   -0.001
## 6 Female preference    Simple  GLMM Beta   -0.204   -0.273   -0.119

##          Phenotype Food_Web Model      Type Estimate lower_2.5 upper_97.5
## 1      Gall diameter    Complex GLMM Quadratic  0.050   -0.052    0.218
## 2      Clutch size     Complex GLMM Quadratic  0.000   -0.186    0.254
## 3 Female preference    Complex GLMM Quadratic  0.032   -0.078    0.146
## 4      Gall size       Simple  GLMM Quadratic  0.090   -0.078    0.266
## 5      Clutch size     Simple  GLMM Quadratic  0.032   -0.070    0.156
## 6 Female preference    Simple  GLMM Quadratic  0.204   -0.008    0.446
```

```

##      Phenotype Food_Web Model          Type Estimate lower_2.5 upper_97.5
## 1 Diam,Clutch  Complex  GLMM Correlational -0.028   -0.085    0.076
## 2 Diam,Pref   Complex  GLMM Correlational -0.104   -0.274    0.074
## 3 Clutch,Pref Complex  GLMM Correlational  0.004   -0.109    0.063
## 4 Diam,Clutch Simple   GLMM Correlational -0.106   -0.215    0.029
## 5 Diam,Pref   Simple   GLMM Correlational  0.003   -0.064    0.097
## 6 Clutch,Pref Simple   GLMM Correlational -0.021   -0.156    0.045

```

Results

We found that more phenotypic traits were under selection in the simple vs. complex food web. In both complex and simple food webs, gall diameter was under strong directional selection, with larger galls resulting in higher larval survival (complex Beta = ; simple Beta =)(Fig. 2A). In complex food webs, there was no evidence of selection on clutch size ($\beta_{clutch} =$) or female preference ($\beta_{preference} =$)(orange lines in Fig. 2B,C). In simple food webs, however, clutch size and female preference were under strong directional selection, with smaller clutch sizes and weaker preferences resulting in higher larval survival (blue lines in Fig. 2B,C). These different selection pressures resulted in different adaptive landscapes in complex vs. simple food webs, with evidence for more rugged landscapes in the simple rather than the complex food web (Fig. 3). Depending on the trait combinations used to create the landscape, we found that the ruggedness of the adaptive landscape ranged from 10% higher (Fig. 3A) to 274% higher (Fig. 3C) in the simple vs. complex food web. Our model comparison suggested that it was unnecessary to test for the effects of non-linear or correlational selection gradients (ref. supp. mat.).

```

##              df      AIC
## beta_control 7 809.1027
## quad_control 10 814.3022
## corr_control 13 817.6910

##              df      AIC
## beta_control      7 809.1027
## glm.beta_control 4 881.0152

##              df      AIC
## beta_control      7 809.1027
## beta_control.REonlyGallID 5 816.4817

##      chisq      ratio      rdf          p
## 349.7811634  0.5025591 696.0000000  1.0000000

##              df      AIC
## beta_treatment 7 619.1030
## quad_treatment 10 620.1640
## corr_treatment 13 622.3247

##      chisq      ratio      rdf          p
## 190.3351784  0.3215121 592.0000000  1.0000000

```

```

##          df      AIC
## beta_treatment    7 619.1030
## glm.beta_treatment 4 712.4146

##          df      AIC
## beta_treatment    7 619.1030
## beta_treatment.REonlyGallID 6 669.5617

## Data: treatment_df
## Models:
## beta_treatment.REonlyGallID: gall_survival ~ sc.gall_size + sc.clutch_size + sc.female_preference +
## beta_treatment.REonlyGallID: (1 | Genotype/Plant_Position)
## beta_treatment: gall_survival ~ sc.gall_size + sc.clutch_size + sc.female_preference +
## beta_treatment: (1 | Genotype/Plant_Position/Gall_Number)
##          Df      AIC      BIC logLik deviance Chisq
## beta_treatment.REonlyGallID 6 669.56 695.93 -328.78   657.56
## beta_treatment              7 619.10 649.87 -302.55   605.10 52.459
##          Chi Df Pr(>Chisq)
## beta_treatment.REonlyGallID
## beta_treatment              1 4.394e-13 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

## Data: treatment_df
## Models:
## glm.beta_treatment: gall_survival ~ sc.gall_size + sc.clutch_size + sc.female_preference
## beta_treatment: gall_survival ~ sc.gall_size + sc.clutch_size + sc.female_preference +
## beta_treatment: (1 | Genotype/Plant_Position/Gall_Number)
##          Df      AIC      BIC logLik deviance Chisq Chi Df
## glm.beta_treatment 4 712.41 730.00 -352.21   704.41
## beta_treatment      7 619.10 649.87 -302.55   605.10 99.311      3
##          Pr(>Chisq)
## glm.beta_treatment
## beta_treatment      < 2.2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

```

Discussion

More recently, researchers have begun to explore how the community context drives evolutionary change (Mcpeek (2017); terHorst et al. (2018)).

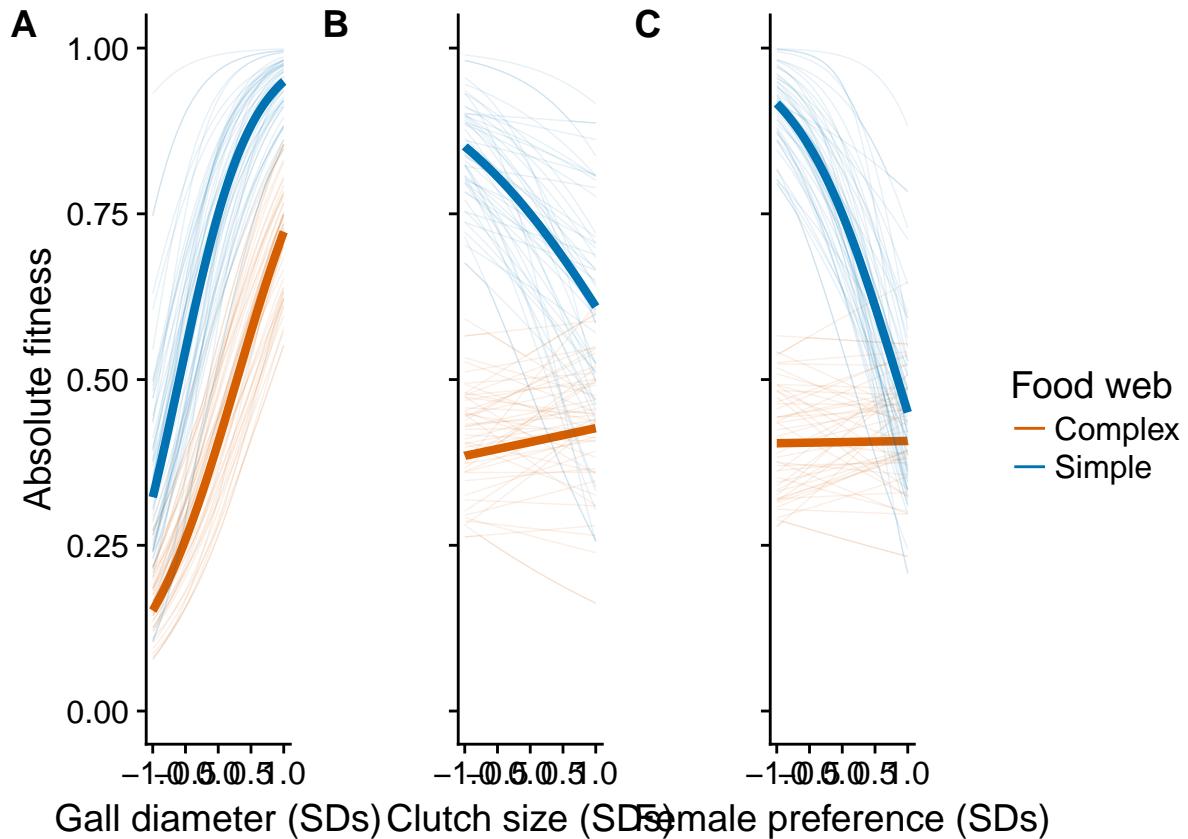
NEED to recognize that evolutionary biologists have begun to explore how community context affects evolutionary change (work by Sharon Strauss, Casey terHorst, Lutz Becks, etc.). These results have begun to show interesting patterns whereby the composition of species in a community can alter the direction and strength of natural selection imposed on species embedded within these communities (cite).

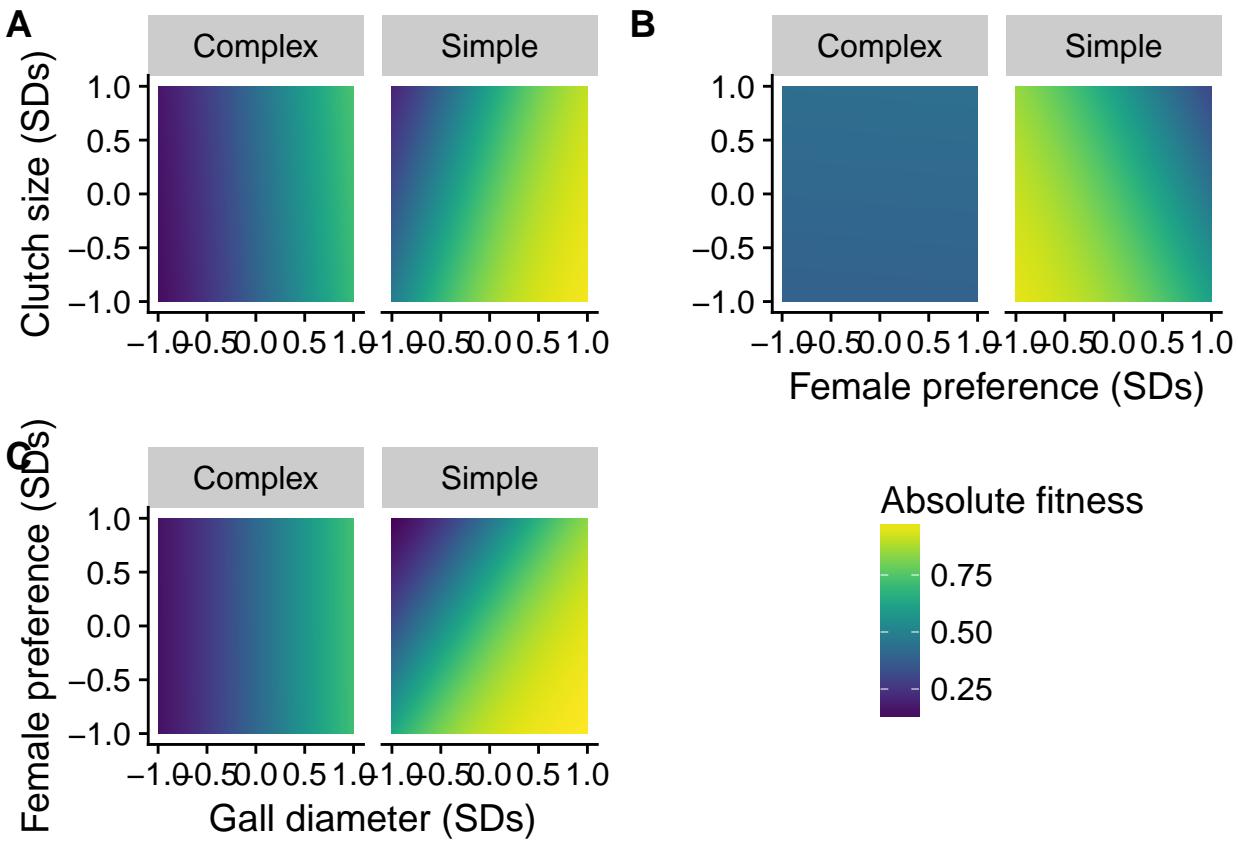
In other words, these results have begun to illustrate show how biological diversity, in terms of differences between species, can shape evolution. Nevertheless, predicting the how the composition of species in a community requires moving beyond a description of community composition. simply knowing the composition of species in a community will

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Here are two sample references: (??; ??).

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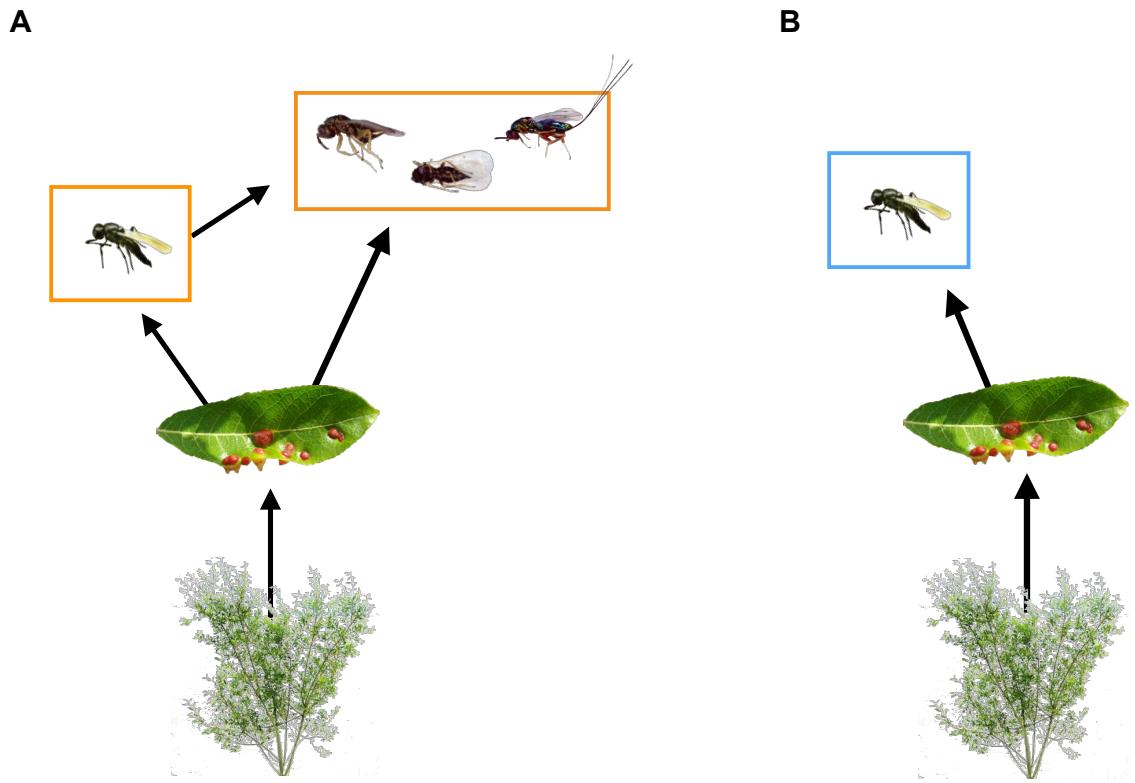


Figure 1: Illustrations of complex (A) and simple (B) food webs associated with the insect herbivore, *Iteomyia salicisverruca*.

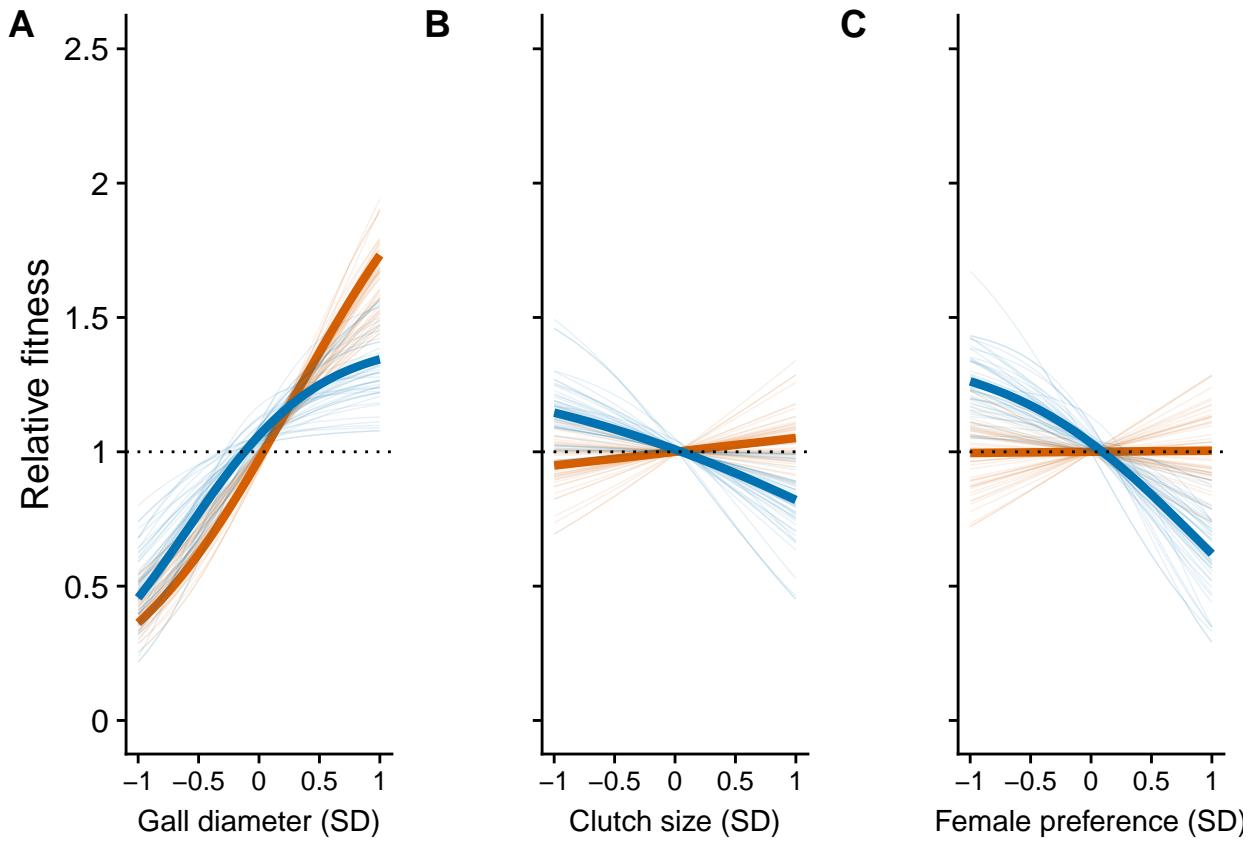


Figure 2: Selection gradients acting on gall traits in complex vs. simple food webs. Each panel corresponds to a different gall trait: gall diameter (A); clutch size (B); and female preference (C). Solid lines represent the estimated gradients in complex (orange) and simple (blue) food webs. Transparent lines represent bootstrapped replicates ($n=100$) to show the uncertainty in estimated gradients.

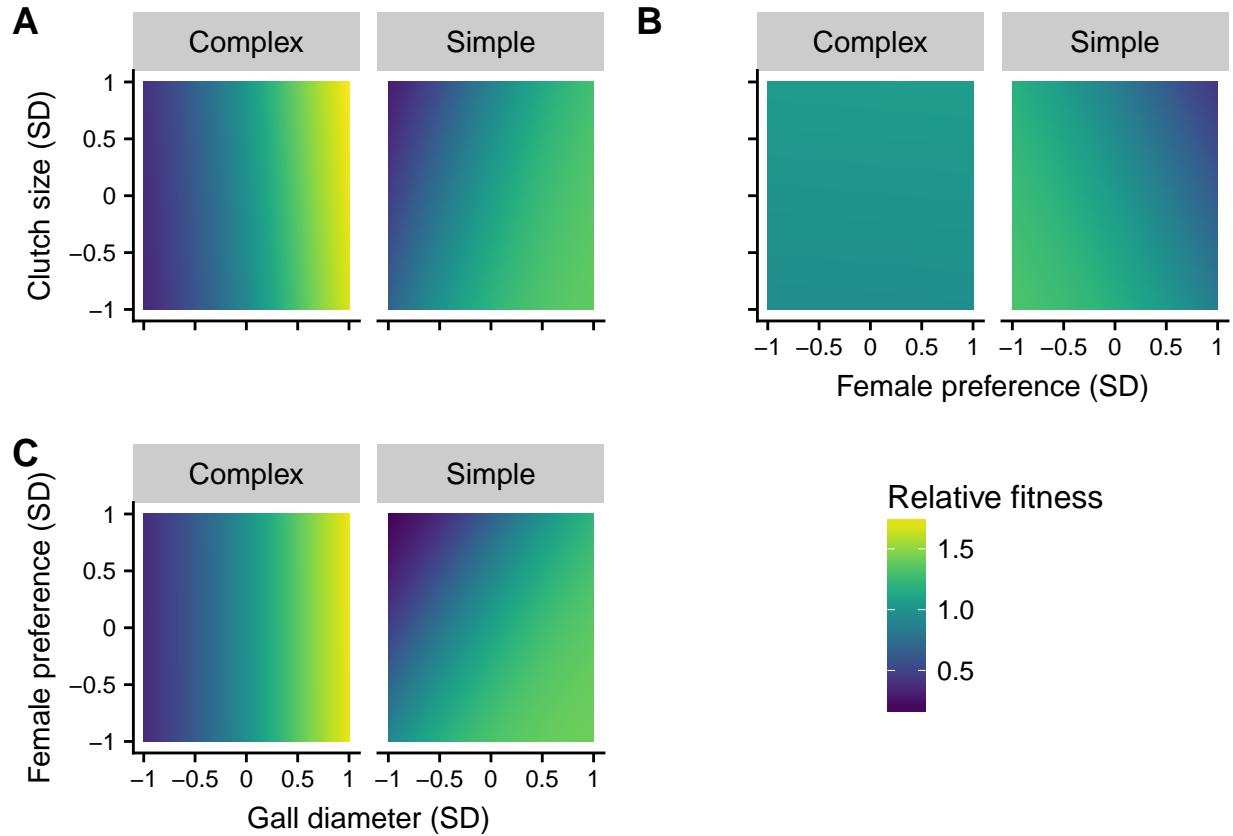


Figure 3: Relative fitness landscapes of gall traits in complex vs. simple food webs. Each panel corresponds to a different combination of traits: clutch size and gall diameter (A); clutch size and female preference (B); female preference and gall diameter (C). Note that traits for all plots range 1 SD below and above the mean ($=0$).