

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

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Abstract

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Introduction

Biological diversity – from genes, to phenotypes, to species – has fascinated evolutionary biologists for decades. Much of this biological diversity has been shaped by natural selection via trophic interactions, such as resource competition (cite charater displacement), mutualistic exchanges of resource (cite?), and predation (cite recent Heath and Stireman paper).

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).

Put another way, these results have begun to show biological diversity, in terms of differences between species, can drive evolutionary change.

While there is clear evidence for pairwise trophic interactions to drive evolution by natural selection, its unclear how biological diversity itself imposes natural selection and drives evolutionary change.

Exploring the effects of biological diversity requires an explicit examination of the network structure of trophic interactions between species in a community. Theoretical models have begun to examine how the network structure of species interactions drives evolutionary change (Nuismer paper; Guimeras paper; Ecology Letters paper from a spanish guy...); however, we are currently lacking experimental tests.

In contrast, ecologists have begun to embrace the complexity of the natural world, and seeking to identify the complex networks of interactions that underlie community structure and ecosystem function. However, these studies have not examined how evolutionary processes feedback to shape the structure and evolution of these interaction networks.

Food-web complexity may influence selection gradients in at least two ways. First, if more diverse predator communities are more efficient as suppressing prey densities (e.g. biodiversity-ecosystem fuction; Ives 2005 Ecology Letters), then this will result in lower mean fitness. A reduction in mean fitness, all else equal, will intensify natural selection and thus could increase the rate of evolutionary change. Alternatively, if predators are functionally distinct, more diverse communities can reduce the strength of selection. This is because each predator has a different functional relationship between prey phenotype and the probability of an interaction.

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Here, we conducted a field experiment to test the effect of food-web complexity on the fitness landscape of a species embedded within this food web. To do this, we used a common garden experiment with a host plant (*Salix hookeriana*), an abundant herbivore (*Iteomyia salicisverruca*), and the diverse community of insect parasitoids that attack it. Prior work in this system has shown that there is directional selection for larger galls, likely because larger galls provide more of a refuge from parasitoid attack. However, there is also evidence that different parasitoid species impose differential selection on gall phenotypes.

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°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 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 (MANUFACTURER 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), 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 identify the appropriate level of model complexity for testing the effects of food-web complexity on the fitness landscapes, we compared models using Akaike Information Criteria. The maximal complexity we explored was a generalized additive mixed model that fit cubic splines to each trait as well as linear interactions between a maximum of two traits as well as an interaction with food-web treatment. We then examined simpler models where

This analysis enabled us to explore the appropriate complexity of the model to include for our analyses (i.e. include non-linear and correlational selection gradients and whether they varied with food-web treatment).

We used generalized additive mixed models (GAMMs, cite Bolker et al. 2008) to test the effects of food-web complexity on the shape of fitness landscape. Larva survival (0 or 1) was our response variable and measure of fitness. We specified our food-web treatment, each gall trait, and all possible statistical interactions, as fixed effects to fully explore the effects of food-web complexity on the fitness landscape. This analysis implicitly assumes that selection is linear, which we felt was a necessary trade-off for exploring the shape of the fitness landscape. We specified plant genotype, plant individual nested within genotype, and multi-chambered gall nested within plant individual, as random effects.

To quantify selection gradients, we fit separate statistical models to data from each food-web treatment. We used the method of Frederic J Janzen and Hal S Stearn (1998) to calculate selection gradients and used parametric bootstrapping to calculate their 95% confidence intervals (Bolker et al. (2009)).

To account for the correlated structure of our gall phenotypes (oviposition preference at plant-level; clutch size at gall-level; gall diameter at chamber-level), we specified gall ID nested within plant ID nested within plant genotype as random intercepts in our statistical models.

From these GAMMs, we estimate selection gradients by assuming the mean value of our random effects (i.e. setting them to zero). This was appropriate for our analysis, since we were interested in estimating the fitness landscape, which is function of population mean fitness and mean trait values.

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.).

Discussion

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