Short Paper

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Abstract

Studies of natural selection and fitness landscapes usually treat the network of interacting species as a "black box". Given that the loss of biodiversity is simplifying the structure of ecological networks, there is a pressing need to answer the question: how does network complexity affect natural selection and the fitness landscape of associated species? To answer this question, we conducted a field experiment that manipulated the complexity of a food web associated with a galling insect herbivore. To maintain complex food webs, we allowed the entire community of natural enemies to attack insect galls on 64 plants in a common garden setting. To create simple food webs, we excluded a guild of three larval parasitoids by bagging galls on 64 different plants; therefore, mortality in this treatment was primarily due to a single egg parasitoid that attacks prior to gall formation. We then measured herbivore survival as a function of three key gall traits in each treatment. We found that more traits were under selection in the simple vs. complex food web. This occurred because different parasitoid species impose different selection pressures on gall traits, thereby minimizing relative fitness differences among insect galls with different phenotypes. Our work suggests that more complex food webs allow phenotypic variation to persist, which could facilitate subsequent adaptive evolution to environmental change.

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Introduction

Biological diversity – from genes, to phenotypes, to species – has fascinated evolutionary biologists for decades. However, the explicit context (i.e. ecological environment) is often abstracted and not examined in detail in studies of natural selection. Thus, we have a poor understanding of how biological diversity itself, imposes natural selection, and the potential feedbacks that may emerge.

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.

We sought to bridge this gap through a field experiment that examines how food-web complexity alters the fitness landscape of species embedded within this food web.

Materials & Methods

To isolate the effects of coastal willow (*S. hookeriana* Barratt ex Hooker) genetic variation on the plant-insect food web, we used a common garden experiment consisting of 26 different willow genotypes (13 males; 13 females), located at Humboldt Bay National Wildlife Refuge (HBNWR) (40°'53"N, 124°12'4"W) near Loleta, California,

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USA. Willow genotypes were collected from a single population of willows growing around Humboldt Bay. While relatedness among these genotypes is unknown, their phenotypes in multivariate trait space are quite distinct from each other (**???**), suggesting that we can treat them as independent from one another. This common garden was planted in February 2009 with 25 clonal replicates (i.e. stem cuttings) of each willow genotype 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 2 - 4 m in height. Further details on the genotyping and planting of the common garden are available in (Barbour et al. 2015).

We conducted our study within a common garden consisting of 26 different willow genotypes (13 males; 13 females), located at Humboldt Bay National Wildlife Refuge (HBNWR) (40°40'53"N, 124°12'4"W) near Loleta, California, USA. Willow genotypes were collected from a single population of willows growing around Humboldt Bay. While relatedness among these genotypes is unknown, their phenotypes in multivariate trait space are quite distinct from each other (Barbour et al. 2016), suggesting that we can treat them as independent from one another. This common garden was planted in February 2009 with 25 clonal replicates (i.e. stem cuttings) of each willow genotype 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 2 - 4 m in height. Further details on the genotyping and planting of the common garden are available in (Barbour et al. 2015). We conducted this experiment across 8 different plant genotypes that span the range of trait variation (Barbour et al. 2015).

We setup our food-web manipulation soon after galls began developing on *S. hookeriana* in early June of 2013. On treatment plants, 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, 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. In late August 2013, we collected marked and bagged galls from each plant. We placed galls in 30 mL transport vials and allowed them to complete development for 4 months at room temperature in the lab. We opened galls under a dissecting scope and determined whether larva survived to pupation, our measure of fitness, or were parasitized.

We collected data on three different phenotypes for each larva that have been shown to be important in our work (cite Barbour et al. 2016) and in other work with Cecidomyiid midges (CITE). First, we measured the size of each gall chamber to the nearest 0.01 mm at its maximum diameter (perpendicular to the direction of plant tissue growth). Second, we counted the number of chambers in each gall, which is indicative of the number of larva per gall. All larva collected from the same gall were scored with the same 'number of larva per gall' phenotype. This trait is indicative of the clutch size of adult females. %This trait likely reflects a combination of both larva feeding behavior as well as adult female preferences and clutch size (CITE Art Weis' work). Third, we estimated gall density as the number of larva per 100 shoots. We did this by counting the number of gall chambers on five randomly sampled branches per tree. To account for potential differences in the number of shoots per branch for each plant genotype (CITE other willow work), we then counted the number of shoots on the fifth branch to estimate the number of larva per 100 shoots for each plant. All larva collected from the same plant were scored with the same gall density phenotype. This phenotype is indicative of the preference of female midges for particular plant traits (hereafter 'female preference').

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 account for the correlated structure of our gall phenotypes (female preference at plant-level; clutch size at gall-level; chamber size 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.

We used a spline-based semiparametric regression (Schluter 1988, Morrissey and Sakedra 2014). These analyses are desirable because they lead to inferences of the form of selection that make few *a priori* assumptions (Schluter 1988), and can now be used to quantify standardized selection gradients (Morrissey and Sakrera 2014).

We then calculated selection gradients as the partial derivatives in absolute fitness (larva survival) with response to multivariate phenotype using the *gsg* package in R (cite Morrissey and R project). We standardized phenotypes (mean=0, SD=1), so that first-order derivatives correspond to the intensity of directional selection (β_{trait}), while second-order derivatives correspond to intensity of nonlinear (γ_{trait}) and correlational selection ($\gamma_{\text{trait}_i,\text{trait}_j}$) and are thus comparable within this study as well as to others.

Our GLMMs are useful for testing the effects of food-web complexity on the fitness landscape within the context of our experimental design; however, coefficients from GLMMs cannot be easily converted into quantitative estimates of selection gradients (cite Morrissey's work). Therefore, for each treatment, we fit separate generalized additive models (GAMs) for gall traits that we identified as being under selection from our GLMMs. Estimating selection gradients from GAMs can give insight to both linear and non-linear selection gradients, which we assumed were all linear for our GLMMs, given the complexity of already fitting up to 4-way interactions in these models. For the number of larva per gall and gall density, we aggregated larva survival at the gall or plant level, respectively, to avoid pseudoreplication in our GAMs.

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References