Density-dependent selection closes an eco-evolutionary feedback loop in the stick insect *Timema cristinae*

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Empirical demonstrations of feedbacks between ecology and evolution are rare. Here, we used a field experiment to test the hypothesis that avian predators impose density-dependent selection (DDS) on *Timema cristinae* stick insects. We transplanted wild-caught *T. cristinae* to wild bushes at 50:50 cryptic:conspicuous morph ratio and manipulated density by transplanting either 24 or 48 individuals. The frequency of the conspicuous morph was reduced by 73% in the low-density treatment, but only by 50% in the high-density treatment, supporting a hypothesis of negative DDS. Coupled with previous studies on *T. cristinae*, which demonstrate that maladaptive gene flow reduces population density, we support an eco-evolutionary feedback loop in this system. Furthermore, our results support the hypothesis that predator satiation is the mechanism driving DDS. We found no effects of *T. cristinae* density on the abundance or species richness of other arthropods. Eco-evolutionary feedbacks, driven by processes like DDS, can have implications for adaptive divergence and speciation.

1. Introduction

Eco-evolutionary feedbacks occur when the evolutionary trajectory of a population is modified by a change in ecology that is itself the result of rapid evolutionary change [1,2]. Despite their detection being a clear goal of current evolutionary ecology research, empirical demonstrations of eco-evolutionary feedbacks are very rare [1,3–6].

The recent focus on eco-evolutionary feedbacks has sparked renewed interest in density-dependent selection (DDS), which occurs when the strength, direction or shape of natural selection changes with population density [4,7]. Because rapid evolutionary change is known to influence population density in some systems [8–10], DDS is a potential mechanism of eco-evolutionary feedbacks [4,11]. For example, in a population for which natural selection increases population density and for which positive DDS is operating (i.e. the strength of selection increases with population density), an eco-evolutionary feedback loop could reinforce adaptation. Accordingly, some recent empirical work has evaluated DDS in this context [5,12,13].

Here, we hypothesize the existence of an eco-evolutionary feedback in the stick insect *T. cristinae* that is driven by DDS. Specifically, we predict positive DDS to occur in accordance with optimal foraging theory [14], whereby birds forage more intensely in patches of high density and cause a greater reduction in the abundance of poorly camouflaged genotypes. Combined with previously published work showing how natural selection reduces *T. cristinae* population density [10], evidence for DDS in this system would suggest that an eco-evolutionary feedback loop is operating.

In this paper, we detect negative DDS, whereby increased population density leads to a reduction in the strength of natural selection, and we test two competing hypotheses to explain this pattern. The predator-satiation hypothesis suggests that negative DDS occurs because predators eat a constant amount of prey,
regardless of density. The search-image hypothesis suggests that negative DDS occurs because predators are more likely to detect cryptic prey at high density, and thus develop a search image. The predator-satiation hypothesis predicts decreased mortality of conspicuous prey at high density, whereas the search-image hypothesis predicts increased mortality of cryptic prey at high density.

Following recent eco-evolutionary research on *T. cristinae*, which shows that maladaptive gene flow reduces arthropod abundance and species richness by attracting avian predators [10], we evaluate whether *T. cristinae* density influences the cohabiting arthropod community. We predict reduced abundance and species richness of caterpillars at the *T. cristinae* density wherever selection is strongest.

2. Material and methods

(a) Study system

*Timema cristinae* is a stick insect endemic to southern California and exhibits a heritable camouflage polymorphism, conferring crypsis against avian predators on different host-plant species: striped individuals are cryptic on *Adenostoma fasciculatum*, whereas unstriped (hereafter, ‘green’) individuals are cryptic on *Ceanothus spinosus* (figure 1 [15]). For an in-depth review of *T. cristinae* natural history and evolutionary biology, see [16].

(b) Experimental set-up

On 17 April 2014, we collected 180 immature striped and 180 immature green *T. cristinae* from a large polymorphic metapopulation, fed them in the laboratory on *Ceanothus* foliage for 52 h, and transplanted them to the field on 19 April. We transplanted 48 and 24 *T. cristinae* at a 50:50 striped : green ratio onto five *Adenostoma* bushes each (10 bushes total), creating high- and low-density treatments in a randomized block design (*n* = 5). All blocks were located within 1 km², but were no less than 50 m apart, and bushes within blocks were between 1 and 5 m apart.

We also collected 100 caterpillars from the same region and added 10 to each bush at the start of the experiment. The caterpillars were distributed across four morphospecies and were evenly distributed across experimental bushes. Six caterpillars of Morphospecies 1 and four caterpillars of three additional morphospecies were added to each bush, yielding four caterpillar species per plant.

To ensure that *T. cristinae* densities were manipulated as intended, through the addition of differential abundance, we trimmed all bushes to 1 m³. Anecdotally, adding 24 or 48 *T. cristinae* individuals yields densities well within the range of naturally occurring populations. Branches of non-experimental plants adjacent to the experimental bushes were removed to reduce immigration and emigration of *T. cristinae*, and each site was sampled twice before transplant to remove any resident *T. cristinae*. Only one *T. cristinae* individual was found during pre-sampling and was not returned to its host plant.

On 23 April, 92 h after transplant, we sampled the experimental bushes by hitting all branches with a stick and collecting fallen individuals in a sweep net. The surrounding area was covered with a white bed sheet to ensure that the maximum number of fallen individuals was recovered. *Timema* morphs and caterpillar abundances per bush were recorded, and every site was subsequently resampled after 5 h to collect individuals that had escaped initial capture by dropping to the base of the plant.

(c) Data analysis

Graphical data exploration clearly showed one severe morph-frequency outlier (electronic supplementary material, figure S1). We found that selection on this bush was extreme relative to four previously conducted selection experiments in *T. cristinae* (one-sample *t*-test, s.e. = 0.037, *t*₁₇ = 5.87, *p* < 0.001; see the electronic supplementary material). Therefore, we performed all analysis of morph frequency both with and without the entire block to which this bush belonged (block 1; see the electronic

![Figure 1. *Timema cristinae* morphs and their respective host-plant species. (a) Striped, (b) green, (c) *A. fasciculatum*, (d) *C. spinosus*. Illustrations credit Rosa Marin Ribas. Adapted from [10] under licence from Elsevier publishing. (Onlin](http://rsbl.royalsocietypublishing.org/)
supplementary material). Results of analyses excluding block 1 are presented in the main text below. Results of analyses including block 1 are presented in the electronic supplementary material and show contrasting results.

To evaluate whether selection had occurred in the experiment, we used a one-sample t-test on raw frequency data, testing the mean final green-morph frequency against a null value of 0.5 to reflect initial morph frequency. To evaluate the effect of Timema density on the frequency of the green morph (i.e. selection), we used a linear mixed-effects model with block as a random factor (see the electronic supplementary material for model validation). Similarly, to distinguish between the predator-satiation and search-image hypotheses, we used two linear mixed-effects models, testing for an effect of T. cristinae density on the proportional reduction in abundance of the striped and green morphs (% morph-specific mortality). To evaluate the effect of T. cristinae density on caterpillar abundance and species richness, we used generalized linear mixed-effects models with Poisson error and block as a random factor (see the electronic supplementary material).

3. Results

In accordance with prior work on T. cristinae [10,15,17], we found strong evidence of natural selection, with a 61% reduction in the frequency of the green morph in only 92 h after experimental transplant (one-sample t-test; s.e. = 0.050, t = 7.45, p < 0.001). Contrary to our expectation of positive DDS, we detected negative DDS in our experiment, with a 73% reduction in green-morph frequency in the low-density treatment, but only a 50% reduction in the high-density treatment (p = 0.022; table 1 and figure 2a). The negative effect of density on the strength of selection was driven by a larger reduction in the abundance (i.e. greater mortality) of the green morph at low density (p = 0.014; figure 2b and table 1), rather than a larger reduction in the abundance of the striped morph at high density (p = 0.718; figure 2b and table 1).

Timema cristinae density did not influence caterpillars. There were no significant effects of density on total caterpillar abundance (p = 0.496, table 1), abundance of caterpillar Morphospecies 1 (p = 0.786, table 1) or caterpillar species richness (p = 0.683, table 1).

4. Discussion

Our results provide clear experimental evidence for negative DDS in T. cristinae (figure 2b and table 1). Coupled with results of another study in this system [10], our results further provide evidence for an eco-evolutionary feedback, mediated by population density. In this feedback, gene flow causes the evolution of camouflage maladaptation in nature, and selection against the conspicuous morph subsequently causes the evolution of well-adapted populations, reducing population density in the process [10]. Our results suggest that density reductions should increase the strength of selection, closing the eco-evolutionary feedback loop.

We distinguish between two possible mechanisms for negative DDS in the context of predator–prey interactions, which have rarely been investigated [18]. Although we do not have a direct measure of predator satiation, we lend support to the predator-satiation hypothesis by demonstrating that the difference in green-morph frequency between density treatments is owing a greater reduction in the proportional abundance of green (conspicuous) individuals at low density.
could investigate the role of the DDS-driven eco-evolutionary feedback in adaptive divergence and speciation.

Lastly, while camouflage maladaptation in *T. cristinae* has been shown to affect the abundance and species richness of cohabiting arthropods, we did not find evidence that *T. cristinae* density influenced the abundance or richness of caterpillars in this study. This finding is not particularly surprising given support for the predator-satiation hypothesis, because this hypothesis posits equal predator visitation between the two density treatments.

**Author contribution.** T.E.F. and G.M.K. designed and performed all field and lab methods. T.E.F. performed data analysis and wrote the manuscript.

**Data accessibility.** Raw data are published in the electronic supplementary material, table S1.

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**References**