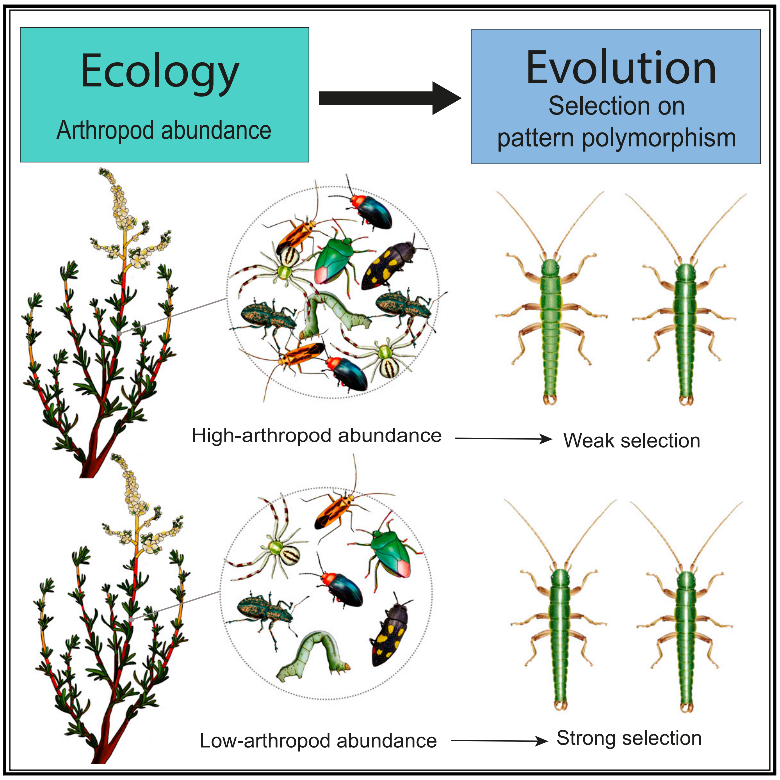
Current Biology

Report

A stabilizing eco-evolutionary feedback loop in the wild

Graphical abstract



Highlights

- Feedbacks between ecology and evolution are challenging to demonstrate in the wild
- We provide experimental evidence of such eco-evolutionary feedback loops in the wild
- Low-arthropod abundance leads to strong selection on crypsis in a stick insect
- The feedback loop is negative and promotes stability by preventing directional change

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In brief

Reciprocal interactions between ecological and evolutionary processes are a central tenet of eco-evolutionary dynamics but have rarely been demonstrated. Zamorano et al. demonstrate that changes in the arthropod community, driven by stick-insect evolution, can feed back to affect further evolution in the stick insect.

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Report

A stabilizing eco-evolutionary feedback loop in the wild

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SUMMARY

There is increasing evidence that evolutionary and ecological processes can operate on the same timescale[1,2] (i.e., contemporary time). As such, evolution can be sufficiently rapid to affect ecological processes such as predation or competition. Thus, evolution can influence population, community, and ecosystem-level dynamics. Indeed, studies have now shown that evolutionary dynamics can alter community structure[3–6] and ecosystem function.[7–10] In turn, shifts in ecological dynamics driven by evolution might feed back to affect the evolutionary trajectory of individual species.[11] This feedback loop, where evolutionary and ecological changes reciprocally affect one another, is a central tenet of eco-evolutionary dynamics.[1,12] However, most work on such dynamics in natural populations has focused on one-way causal associations between ecology and evolution.[13] Hence, direct empirical evidence for eco-evolutionary feedback is rare and limited to laboratory or mesocosm experiments.[13–16] Here, we show in the wild that eco-evolutionary dynamics in a plant-feeding arthropod community involve a negative feedback loop. Specifically, adaptation in cryptic coloration in a stick-insect species mediates bird predation, with local maladaptation increasing predation. In turn, the abundance of arthropods is reduced by predation. Here, we experimentally manipulate arthropod abundance to show that these changes at the community level feed back to affect the stick-insect evolution. Specifically, low-arthropod abundance increases the strength of selection on crypsis, increasing local adaptation of stick insects in a negative feedback loop. Our results suggest that eco-evolutionary feedbacks are able to stabilize complex systems by preventing consistent directional change and therefore increasing resilience.

RESULTS AND DISCUSSION

Evidence for eco-evolutionary feedbacks in natural settings generally requires experiments, as feedbacks might be difficult to detect with simple observations in systems that are near equilibrium or fluctuate at scales that are more rapid or subtle than those at which scientists tend to take measurements (i.e., eco-evolutionary feedbacks can be "cryptic").[14,17] Therefore, experimental tests in the wild are required to elucidate whether and how feedback loops govern eco-evolutionary dynamics, with predictions as follows.[1,18] Strong positive feedback, which is self-reinforcing, might drive sudden directional change. Weaker positive feedback might result in only gradual change. In contrast, negative feedback might prevent directional trends or dampen cycles driven by other ecological or evolutionary processes, promoting stability. These predictions about change and stability apply broadly, such that feedback loops are potentially important for understanding not only ecology and evolution but also other complex systems across the physical and life sciences.[18,19] Here, we use a field experiment to test for an eco-evolutionary feedback loop in the wild using plant-associated arthropod communities. To do so, we focus on the following: (1) *Timema cristinae*, a common, wingless, plant-feeding stick insect known to undergo rapid evolution driven by natural selection[20] and (2) the arthropod community that co-occurs with this species.

T. cristinae is a good candidate for exploring the presence of eco-evolutionary feedback loops in nature because it plays a key role in mediating prey-predator interactions[4] and manipulative experiments in the wild are possible.[21,22] This species exhibits two highly heritable color-pattern morphs—a striped morph that is cryptic on the host-plant *Adenostoma* and an unstriped morph that is conspicuous on *Adenostoma* but cryptic on another host (*Ceanothus*).[21–23] Past work has shown that rapid evolution of color-pattern morph frequencies in *T. cristinae* occurs and that morph frequencies affect entire arthropod communities through the role that cryptic patterns play in modulating bird predation.[4] Thus, evolution (i.e., morph-frequency change) is known to affect ecological variables (i.e., arthropod abundance) in this system (i.e., an evo-to-eco path,

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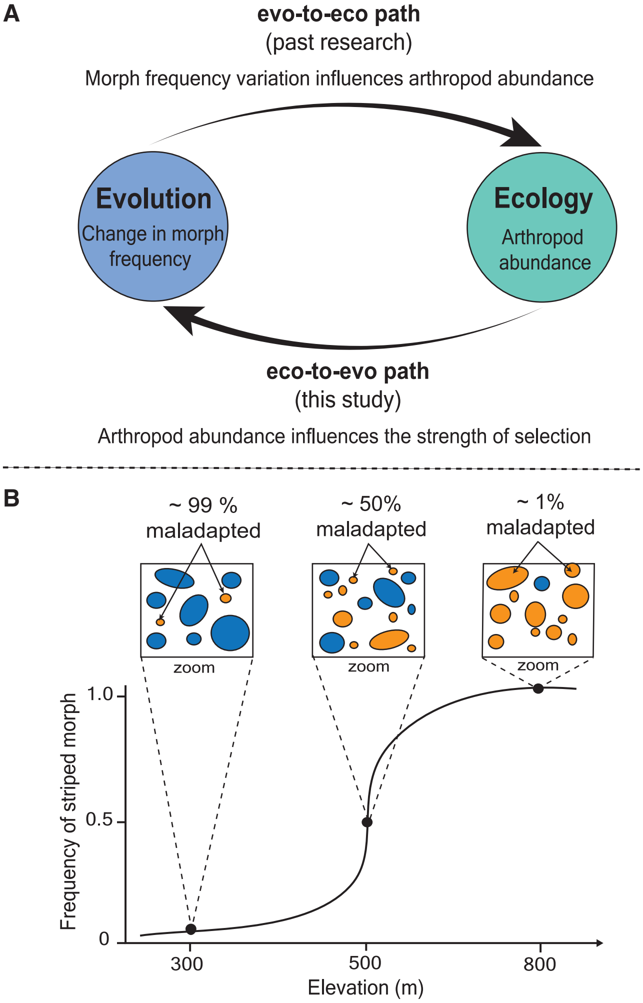

Figure 1. Graphical representation of an eco-evolutionary feedback loop and the maladaptive landscape of *Timema cristinae*

(A) Graphical representation of feedback between evolution (blue circle) and ecology (green circle), generally and in the *T. cristinae* system. Changes in the frequency of color-pattern morphs of *T. cristinae* through natural selection are associated with concomitant changes in the abundance of co-occurring arthropods (i.e., evo-to-eco path). In turn, this change in the arthropod abundance could feed back to affect the strength of selection (i.e., eco-to-evo path).

(B) Graphical representation of the spatial distribution of host plants and the frequency of *T. cristinae* color-pattern morphs across an elevational gradient. The zoomed-in boxes represent the composition of host plants at a given elevation: blue circles correspond to *Ceanothus spinosus* plant individuals, and orange circles represent *Adenostoma fasciculatum* individuals. *Adenostoma* bushes are more frequent at higher elevations in comparison with *Ceanothus*, and vice versa. As a result, the percentage of striped *T. cristinae* tends to increase with elevation due to changes in host-plant distribution. At lower elevation, populations are dominated by *Ceanothus* host plants; hence, the frequency of striped individuals is low (e.g., ∼1%). In this scenario, isolated patches of *Adenostoma* have a high level of maladaptation due to gene flow (e.g., ∼99% of individuals can be maladapted). At medium elevation, where there is a similar proportion of both host-plant species, the average frequency of striped individuals is more mixed and variable (e.g., 50% maladaptation). At higher elevation, populations are dominated by *Adenostoma* host plants, with an elevated proportion of striped individuals (e.g., ∼99% of individuals are striped, leading to ∼99% maladaptation on *Ceanothus* rather than *Adenostoma*).

See also Figure S2.

Figure 1A; see the STAR Methods section study system: eco-evolutionary dynamics in the Timema cristinae study system for details). In contrast, whether these ecological changes then feed back to affect further evolutionary change (i.e., an eco-to-evo path) has not been tested in past work. In order to address this issue, here we conduct an experiment that (1) starts by manipulating the endpoint of the dynamic documented in previous work (i.e., communities comprised of few versus many arthropods) and (2) tests whether this variation in arthropod abundance feeds back to affect further selection and evolution in *T. cristinae* (see STAR Methods section for details required to understand our study).

In this context, previous field experiments showed that variation in color-pattern morph frequency in *T. cristinae* causally affects population and community dynamics[4] (e.g., the evo-to-eco path shown in Figure 1A). This past work manipulated local maladaptation of *T. cristinae*, where maladaptation is defined as the proportion of the locally non-cryptic morph (e.g., percent unstriped morph on *Adenostoma*). The results revealed that high local maladaptation was associated with reduced abundance and richness of co-occurring arthropods but only in the presence of bird predation (i.e., this association did not occur in bird exclusion treatments). The collective evidence is consistent with birds foraging on bushes where the frequency of the maladapted morph is high (relative to when it was manipulated to be low), resulting in reduced population density of *T. cristinae* and a concomitant decrease in the abundance and species richness of arthropods.[4]

Notably, these past experiments used fixed morph frequencies on individual experimental bushes (100 versus 0 percent cryptic) and sampled only at a single time point after release. Thus, whether the observed morph-driven ecological changes in the community feed back to affect further evolution in *T. cristinae* is unknown. Here, we experimentally manipulate arthropod abundance to test for such "arthropod-to-*Timema*" or "eco-to-evo" causality, which could close the eco-evolutionary feedback loop in this system by affecting the strength of natural selection on the *T. cristinae* pattern polymorphism. Our experiment thus replicates the endpoint of the dynamic documented in previous work,[4] where evolution, driven by variation in maladaptation, results in communities composed of many versus few arthropods.

The experiment thus involved transplanting many versus fewer arthropods to experimental bushes of *Adenostoma* in the wild (Figure 2A; STAR Methods). Specifically, we transplanted 600 versus 200 non-*Timema* arthropod individuals in "high-abundance" and "low-abundance" treatments, respectively. To these same experimental bushes, we added a mixture of striped and unstriped *T. cristinae*, at a 50:50 ratio to maximize the potential for evolutionary change (40 individuals in total). We recaptured these *T. cristinae* a few days later and scored morph frequencies to infer natural selection and potential evolutionary change. Larger departures from 50:50 frequencies likely result in more evolution, but even in the absence of evolution, strong inferences can be made about the core evolutionary process of natural selection within a generation. Past work using these procedures has shown that the recapture of *T. cristinae* in such experiments is a good proxy for survival, with limited to zero dispersal away from the experimental bushes.[24,25]

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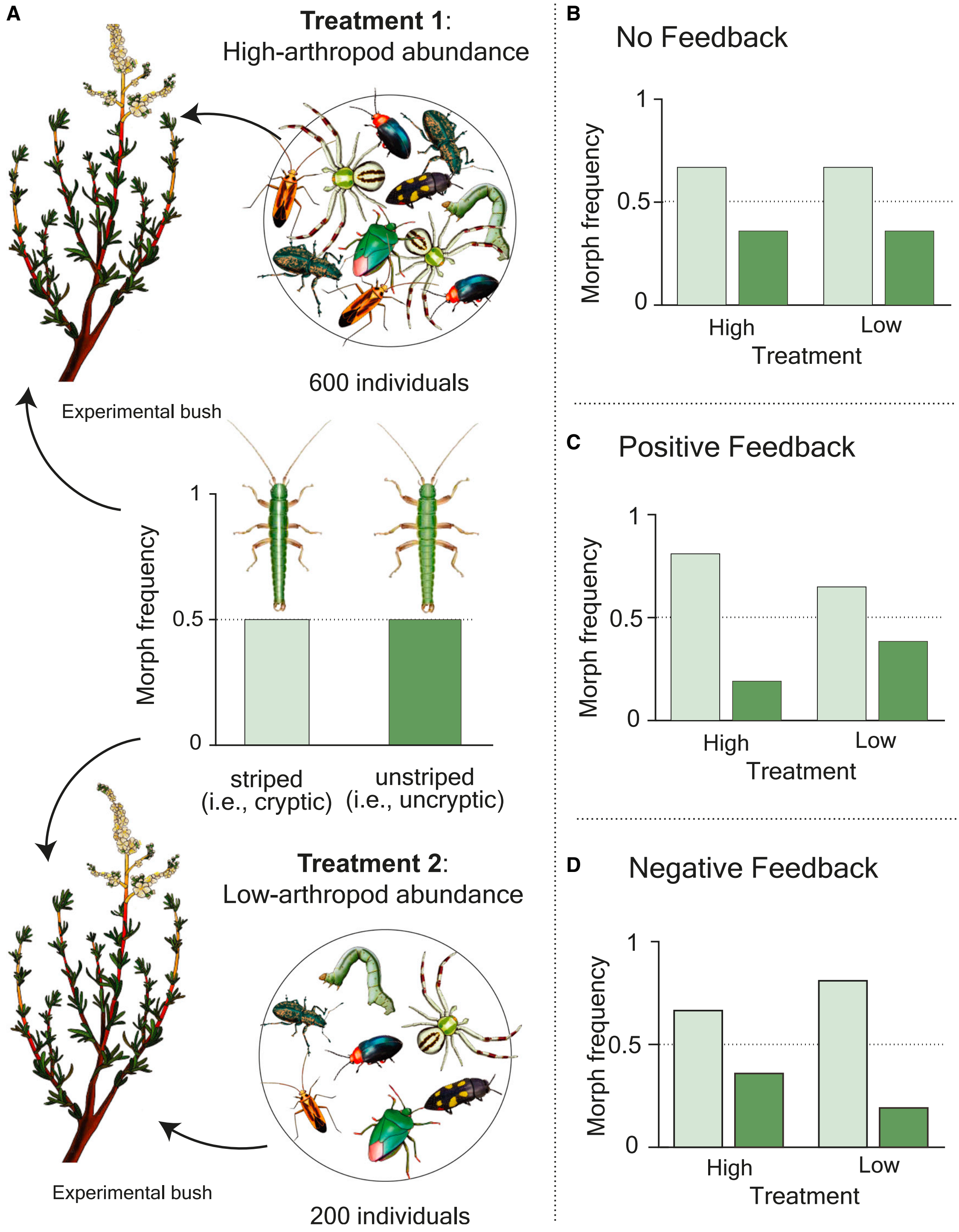

Figure 2. Experimental design and predictions for eco-evolutionary feedback

(A) Schematic description of the experimental design. At time = 0, we transplanted 600 versus 200 arthropod individuals (high-abundance and low-abundance treatments, respectively), onto individual *Adenostoma fasciculatum* bushes in nature in a replicated paired-blocks design (see also Figure S2). At the same time, we transplanted 40 *T. cristinae* individuals to the same bushes with an equal proportion of striped and unstriped morphs. After 3 days (time = 1), we recaptured all the remaining *T. cristinae* individuals to determine changes in morph frequency (i.e., potential selection). We envisaged three potential outcomes in this experiment, predictions are depicted in (B)–(D). “s” stands for striped morph, and “u” stands for unstriped morph.

(B) A scenario involving no feedback; the cryptic striped morph is predicted to increase in frequency independently of the arthropod-abundance treatment. In contrast, if feedback is involved, morph-frequency changes are predicted to depend on the arthropod treatment.

(legend continued on next page)

Our experimental design allowed us to test the hypothesis that arthropod abundance affects selection in *T. cristinae*, closing the eco-evolutionary feedback loop. Predictions were as follows: if morph-frequency changes (i.e., evolution) are independent from treatment, then the feedback hypothesis is unsupported (Figure 2B). For example, the striped morph, being more cryptic on *Adenostoma*, might experience a comparable increase in frequency in both treatments. By contrast, if the feedback hypothesis is supported, two types of feedback are possible: negative and positive. The direction of the feedback is of interest as it might affect the dynamics of the eco-evolutionary system. In the context of our experiment, a positive feedback loop would result in an increase in the frequency of the cryptic morph in the high-arthropod abundance treatment (Figure 1C). Under this scenario, high-arthropod abundance leads to local adaptation in the stick insects, which, in turn, leads to further increased arthropod abundance, in a self-reinforcing cycle that could lead to directional change. In contrast, a negative feedback loop would lead to a higher frequency of the cryptic morph in the low-arthropod abundance treatment (Figure 2D). In turn, strong selection in the low-abundance treatment would increase subsequent adaptation, counteracting the predation that led to low-abundance in the first place and, ultimately, allowing the system to recover toward higher densities (at least during this phase of the fluctuating dynamic).

Variation in arthropod abundance generates a negative feedback loop

Consistent with the feedback hypothesis, the proportion of the striped morph recaptured in our experiment differed between low- and high-abundance arthropod treatments (Figure 3A; Table S1; see specifically Tables S1 and S2 for full recapture counts and details). Specifically, we recaptured a higher proportion of the striped morph in the low-abundance treatment (mean percent striped recaptured = 88% ± 0.08 standard deviation, n = 5 paired blocks) than in the high-abundance treatment (mean percent striped recaptured = 46% ± 0.12, n = 5 paired blocks). In addition, we observed an increase in the frequency of cryptic, striped, individuals between release and recapture in the low-abundance treatment for all five paired blocks (Figure 3B; Table S2), demonstrating consistency of trends in this treatment. In contrast, morph-frequency changes were more idiosyncratic in the high-abundance treatment (Tables S1 and S2). The collective trends resulted in significant differences between treatments (Wilcoxon test: n = 10, t = 2.95, p value = 0.022), with the nature of the differences supporting that the feedback loop would be negative.

To further quantify uncertainty in these results, we modeled recapture probabilities as a function of arthropod density in an explicitly Bayesian framework (see STAR Methods for Bayesian hierarchical generalized model). This revealed that recapture probabilities for the cryptic striped morph were higher when the arthropod abundance was manipulated to be low versus high (h posterior median = −1.52, 95% equal-tail probability interval [ETPI] −2.75 to −0.39; Figure S1).

Variation in arthropod abundance affects the strength of selection

A key feature of our results is non-random mortality with respect to morph (i.e., natural selection). Knowing the strength of selection on the stripe morph and how this varies with arthropod abundance is important for predicting the dynamics of evolutionary change and the associated ecological consequences.[1] We thus next explicitly estimated selection using an approximate Bayesian computation (ABC) approach. Specifically, we set the relative fitness of stripe to 1.0 ($\Omega_{striped} = w_{striped}/w_{striped}$—where w equals to absolute fitness) and defined the relative fitness of unstriped as the ratio of absolute fitness of unstriped to striped ($\Omega_{unstriped} = w_{unstriped}/w_{striped}$). We then defined the selection coefficient, *S*, as the relative-fitness-striped − the relative-fitness-unstriped (i.e., the difference in relative fitnesses; see STAR Methods for details on approximate Bayesian computation). This revealed a strong selection in favor of the striped morph in the low-abundance treatment (*S* posterior median = 0.75, 95% ETPI 0.29 to 0.99; Figures 4A and 4D). By contrast, *S* in the high-abundance treatment was close to zero (*S* posterior median = −0.02, 95% ETPI −0.81 to 0.57; Figures 4B and 4D). Hence, we detected evidence of strong selection in the low-abundance treatment, but no evidence for selection in the high-abundance treatment (Figure 4D). This led to a marked and credible difference in selection between treatments (median difference in *S* = −0.738, ETPI −1.49 to −0.08; Figure 4C). A consequence of such strong selection, given a negative feedback loop, is that we expect rapid change in morph frequencies during some time periods, but a system that is strongly stabilized overall and resistant to consistent directional change.

Notably, despite selection (non-random mortality), overall mortality did not differ markedly between treatments, with 17% and 21% of released *T. cristinae* recaptured in the high- and low-abundance treatment respectively (chi-squared test: $\chi^2 = 1.33$ p = 0.86). Although our data do not allow us to infer the exact causes of this pattern, some plausible mechanisms based on foraging theory are as follows. Predators might spend less time foraging in the low-abundance treatment (i.e., poor patches) to optimize energy gain among patches.[26,27] In this case, they are expected to consume the conspicuous, non-cryptic morph (i.e., unstriped morph) and then move on to new patches before switching to alternative prey items. In contrast, predators might forage longer in the high-abundance treatment resulting in equivalent consumption of both morphs.[28] This process predicts stronger selection and a greater increase in the cryptic morph in the low-abundance treatment, as we observed (Figure 2D). In principle, the efficacy of crypsis and time spent foraging by predators might interact in a manner such that selection (i.e., non-random mortality) is stronger in one treatment, but overall, mortality is comparable between treatments, a topic that requires further research.

(C) With positive feedback, we predict stronger selection when arthropod abundance is high, resulting in a greater increase in the frequency of striped *T. cristinae* in this treatment compared with the low-abundance one.

(D) With negative feedback, we predict stronger selection when arthropod abundance is low, resulting in a greater increase in the frequency of striped *T. cristinae* in this treatment compared with the high-abundance one.

See also Figure S2.

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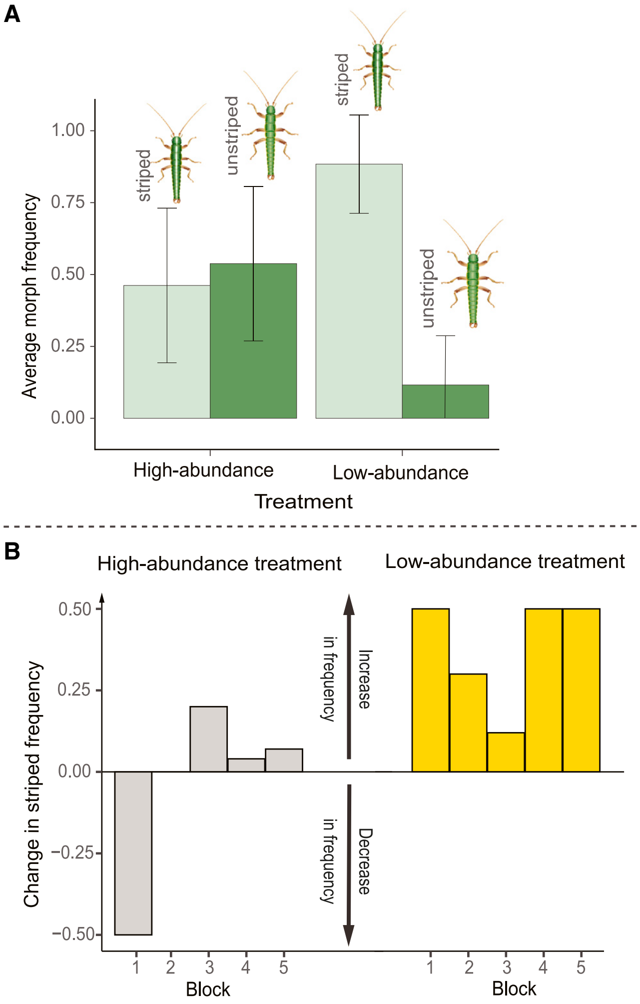

Figure 3. Results of the transplant experiment

(A) Mean frequency of striped (shown with light-green bars) and unstriped (shown with dark-green bars) *T. cristinae* recaptured in high- versus low-arthropod abundance treatments. Error bars depict 95% confidence intervals (see also Table S1).

(B) Changes in the frequency of striped morphs released in high- versus low-arthropod abundance treatments. Bars represent the difference in the proportion of striped individuals (relative to unstriped individuals) between release and recapture in each pair of experimental blocks (% of striped individuals recaptured − % of striped individuals released). The high-arthropod abundance treatment consisted of 600 arthropod individuals, and the low-arthropod abundance treatment consisted of 200 arthropod individuals. There is a substantial increase in the frequency of striped individuals in the low-abundance treatment that is largely consistent across all five blocks. By contrast, changes in the striped morph frequency were less pronounced and more idiosyncratic in the high-abundance treatment. These observations correspond to the scenario of a negative feedback loop as depicted in Figure 2D (see also Figure S1 and Tables S1 and S2).

Eco-evolutionary dynamics in the *T. cristinae* system

Our results provide experimental evidence that the local abundance of non-*Timema* arthropods affects the strength of selection on *T. cristinae*, which closes an eco-evolutionary feedback loop. Even though natural environments are extremely complex, with many covarying factors acting alongside any specific causal factor, the strong and consistent increases in the frequency of the striped morph observed under the low-arthropod abundance treatment are almost certainly related to the experimental treatment. In other words, even if many other factors are likely to be at play, we experimentally manipulated arthropod abundance in a replicated fashion such that these uncontrolled factors are unlikely to systematically differ between treatments and explain our results. We further note that past work in the *T. cristinae* system has shown that unperturbed morph frequencies do not fluctuate strongly over the timescale of our experiment,[20,22,29] and thus our experimental perturbation likely contributed to the observed morph-frequency changes. Finally, the selection strength calculated in our experiment is comparable to the selection strength observed in other studies in the *Timema* system[21,22,24,30] as well as other systems with strong and fluctuating selection.[31,32]

The dynamic interplay between evolution and ecology documented here is of broad biological interest because it moves beyond pairwise co-evolutionary processes to more complex species interactions that can affect emergent community-level processes such as stability. More precisely, we show that high-arthropod abundance results in a relaxation of selection such that both morphs of *T. cristinae* experience similar survival rates. This could cause local maladaptation, for example via strong effects of migration and gene flow between populations on different hosts. Past work showed how a rise in maladaptation enhances bird predation and reduces arthropod abundance.[4] Our results predict this would, in turn, generate strong selection thereby completing a negative feedback loop. Based on these collective results, we suggest that this negative feedback loop should generate a fluctuating dynamic but one that promotes longer-term evolutionary and ecological stability in the arthropod system.

In conclusion, our study represents an important and generally lacking experimental demonstration of an eco-evolutionary feedback loop in the wild. In addition, the negative direction of the feedback provides support for the hypothesis that cryptic eco-evolutionary feedback loops are potentially more ubiquitous than expected and can be important for the maintenance of equilibrium in natural systems. Specifically, our results show that the interplay between ecology and evolution may be key in stabilizing complex ecological systems by increasing resistance to consistent directional change. This means that the long-term magnitude of evolutionary (i.e., phenotypic or genetic) and ecological change may often be modest in natural systems, and when changes do occur, they are quickly offset such that the system returns to its previous state,[14,18] unless it loses resilience and tips between alternative states.[33,34] Thus, "cryptic eco-evolutionary feedback loops" might be important in regulating ecological systems. However, our study does not take into consideration all aspects of the fluctuating dynamics of arthropod abundance and the nuances of bird predatory behavior. Further experiments and observational data that more fully account for such interactions in both space and time are required to enhance our understanding of the general prevalence and importance of feedback for the resilience of eco-evolutionary systems. In turn, such information might be relevant for understanding the dynamics of other complex systems across the life and physical sciences.[18]

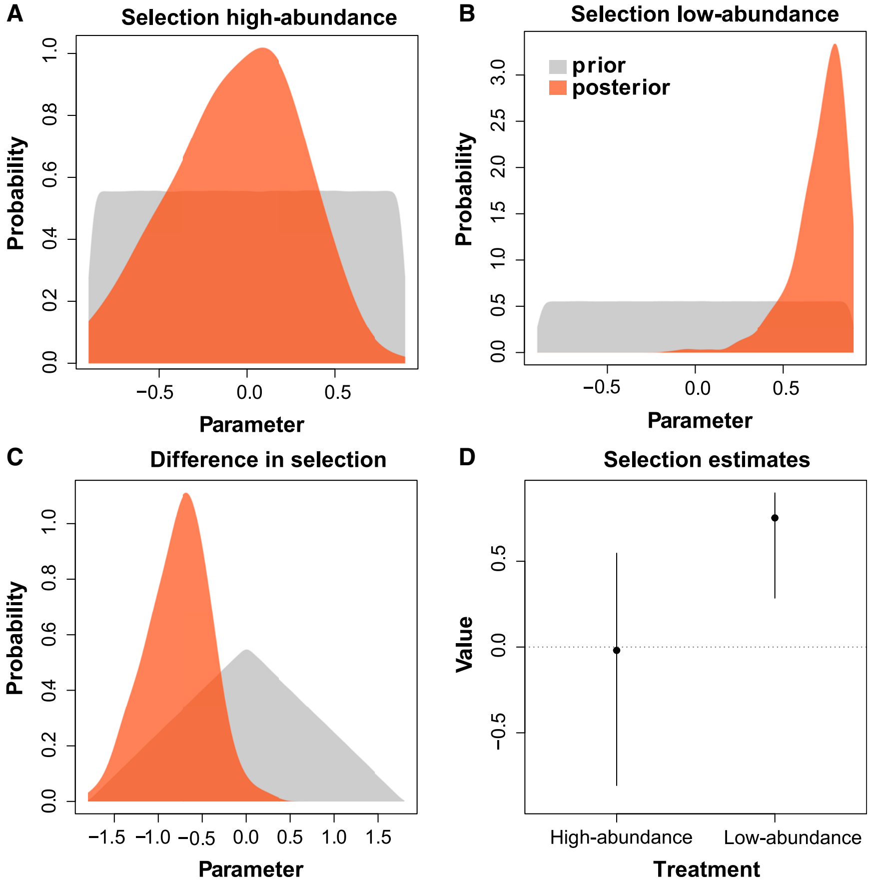

Figure 4. Prior and posterior probability distributions of selection coefficients in high- and low-arthropod abundance treatments

Posterior parameter densities are indicated by an orange curve; prior parameter densities are indicated by a gray curve. Shaded areas correspond to prior or posterior distributions; the vertical line shows the mean posterior probability.

(A) Selection coefficient for high-abundance treatment: median $S = -0.02$, equal-tailed probably intervals, 95% ETPI -0.081 to 0.57.

(B) Selection coefficient for low-abundance treatment: median $S = 0.75$, 95% ETPI 0.29 to 0.99.

(C) Selection coefficient difference between low- and high-arthropod abundance treatment (S-high $-$ S-low): median difference in $S = -0.738$, ETPI -1.49 to -0.08.

(D) Selection coefficient (S) for high- and low-arthropod abundance treatment; dots denote point estimates (posterior median); vertical lines represent 95% ETPI for S_{low} and S_{high}, respectively.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2023.06.056.

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AUTHOR CONTRIBUTIONS

L.S.Z. and P.N. conceived the project and collected data from the experiment. L.S.Z., P.N., and Z.G. contributed data analysis. L.S.Z. and P.N. wrote the manuscript with feedback from all the authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
feedback_experiment_042022.csv	Dryad	https://doi.org/10.5061/dryad.bk3j9kdj1
README.md	N/A	N/A
Software and algorithms		
code	Dryad	https://doi.org/10.5061/dryad.bk3j9kdj1

RESOURCE AVAILABILITY

Lead contact

Further information and request required to reanalyze the data reported in this paper should be directed and will be fulfilled by the lead contact, Laura S. Zamorano (laura.s.zamorano@gmail.com).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper and original code has been deposited at Dryad and is publicly available as of the date of publication. DOIs are listed in the key resources table.
- All original code has been deposited at Dryad and is publicly available as of the date of publication. DOIs are listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request."

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study System: Eco-evolutionary dynamics in the *Timema cristinae* study system

Timema cristinae is a very abundant, flightless, folivorous stick insect (Order: Phasmatodea) endemic to the Santa Inez Mountains in southern California. It occurs primarily on two host-plant species: *Adenostoma fasciculatum* (Rosaceae) and *Ceanothus spinosus* (Rhamnaceae).[22,35,36] *Timema cristinae* exhibits a host-plant associated polymorphism characterized by the presence versus absence of a white longitudinal dorsal stripe (i.e., striped and unstriped morphs, respectively, the 'pattern polymorphism' hereafter).[22,35,36] These morphs are subject to natural selection through bird predation and are thus divergently adapted to the two host plants, with striped individuals being more cryptic on *Adenostoma* and unstriped individuals more cryptic on *Ceanothus*.[21,35] This conclusion is supported by spatial patterns of morph frequency variation but also by the fact that past experiments have reported divergent natural selection on color-pattern in the presence but not in the absence of bird predators,[21,25] and in controlled trials with trained bird predators.[22] Thus, bird predation is a major source of natural selection on this polymorphism. In addition to the green striped and unstriped morphs, *T. cristinae* exhibits a brownish-grey 'melanistic' morph that is cryptic against plant stems and soil.[22,23,36] However this morph is relatively rare and does not differ systemically in frequency between host-plant species, and was thus not included in this study.

Despite strong natural selection for crypsis, both striped and unstriped morphs are retained as a balanced polymorphism within host species, in large part due to ongoing gene flow between populations on different hosts.[30,36] Thus, morph frequencies in nature vary in a mosaic pattern determined by the balance between selection and gene flow. This results in morph frequencies varying from unstriped-dominated populations, to mixed populations, to striped-dominant populations, following an elevational gradient that correlates with the host-plant distribution[22,37] (Figure 1B). In addition, genetic and genomic studies have demonstrated high heritability and major locus control of the pattern polymorphism in *T. cristinae*, such that phenotypic changes in morph frequency very likely result in genetic evolution.[19,23] Specifically, between-generation shifts in morph frequencies, and even allele frequency shifts at the genetic region underlying color-pattern, have been documented in several studies such that evolution of morph frequencies takes place on contemporary timescales.[20,29,30] Evolution aside, natural selection within generations is also readily measurable through observable changes in morph frequency.[21,29] The details above show that the *T.cristinae* system is poised for testing eco-evolutionary dynamics in the wild.

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METHOD DETAILS

Experimental design

We conducted an experiment to test whether variation in the arthropod community affects selection on *T. cristinae* cryptic morphs. In this context, we manipulated the abundance of arthropods into high- and low-abundance treatments on individual host bushes of *Adenostoma fasciculatum* in nature. We then transplanted *T. cristinae* to the same bushes in a 50% ratio of striped and unstriped individuals. We allowed the experiment to run for three days in natural conditions after which we resampled each plant to determine the total number of *T. cristinae* individuals recaptured and changes in the proportion of each morph (See Tables S1 and S2 for recapture data).

To do this, we collected *T. cristinae* and arthropods using sweep nets in the Santa Ynez Mountains, near Santa Barbara, California, USA in March 2022. A total of 200 *T. cristinae* and 4000 arthropod individuals were collected between the 6th and 14th of March 2022; all from *Adenostoma* host plants. The arthropods were collected near and around the eventual transplantation site, with the goal of minimizing the extent to which they were being moved while still allowing a sufficient number of them to be collected for the experiment. The total sampling area thus entailed an area of a few square kilometers near 34’51126 N, 119.79861 W (Figure S2). The area corresponds to an open chaparral habitat at an elevation of 840 m. The habitat is dominated by *Adenostoma* host-plants with sparse *Ceanothus* individuals. All arthropod specimens were kept alive in large plastic containers and transported to a field laboratory where they were counted and sorted into groups of approximately 200 individuals. These groups were then isolated into separate containers. *Timema* individuals were scored for color-pattern (unstriped versus striped) following past work[2–4,19,20] and kept separately in plastic containers.

We used a replicated, randomized block design with two treatment levels: high-abundance (600 arthropod individuals) and low-abundance (200 arthropod individuals). Based on past data on the natural per-bush abundance of arthropods,[38] the abundances used here were high, but not totally unrealistic. Each of the two treatments was replicated five times within the study site. Experimental bushes were chosen to be approximately the same size and have a similar gross light exposure. The area below and surrounding the experimental bush was cleared out of other vegetation in order to minimize dispersal of arthropods to or from adjacent plants. Thus, we created ‘experimental islands’ such that any change in density or morph frequency of *T. cristinae* was assumed to be from mortality, as strongly suggested by past work.[24,25,38] Our experimental design implements a complete randomized block design which allowed us to account for possible sources of error intrinsic to field experimentation (e.g., differences in light exposure, micro-climate etc). Such variations might occur but should be random with respect to treatment such that they cannot readily explain treatment effects.

On March 15, 2022, we transplanted 40 *T. cristinae* individuals to each treatment in each block. Because we were interested in whether arthropod abundance has an effect on color-pattern morph frequency in *T. cristinae*, we used a 50% ratio of striped and unstriped individuals, thus maximizing the potential for evolution and selection. In parallel, the arthropods corresponding to each treatment were transplanted to the experimental bushes. To ensure that the arthropods were transferred to the bushes, we placed the open plastic containers in the middle of the plant and let the arthropods move from the plastic container to the plant undisturbed. A schematic description of the experiment is presented in Figure 2, and a map of the experimental area is presented in Figure S2.

Past studies in *T. cristinae* have documented adaptive divergence between experimental populations within days upon transplantation, with bird predation being a major source of selective mortality.[21,25,31] Hence, we let the experiment run for three days after which we recaptured the surviving individuals using sweep nets and examining each experimental bush. Each branch of the experimental bush was systematically sampled for *T. cristinae* individuals. Sampling ceased when no new individuals were collected after at least several minutes of extensive searching. In addition, the total number of *T. cristinae* recaptured on each experimental bush and the color-pattern of each individual were recorded.

QUANTIFICATION AND STATISTICAL ANALYSIS

Bayesian hierarchical generalized model

We used a Bayesian hierarchical generalized model to determine whether a difference in arthropod abundance affected the survival probability of each morph. This allowed us to test whether the recapture frequency of the cryptic morph differed between treatments. To do this, We assumed that the recapture striped count for bush $\boldsymbol{i}$ was $y_i \sim$ binomial (p_i, n_i), where n_i is the total number of *T. cristinae* individuals recaptured and p_i is the proportion of recaptured individuals that were striped (i.e., cryptic on our experimental host-plant, *Adenostoma*). We defined a linear model for logit(p_i) as logit(p_i) $=\theta_j + \beta * x_i$. Here, θ_j is a block effect (for block j), $x_{\boldsymbol{i}}$ is a binary (dummy) variable denoting the treatment (0 = low-abundance, 1 = high-abundace) and β is the treatment effect. We placed a (relatively uninformative) normal prior on beta, normal(mean = 0, standard deviation [sd] = 10). We used hierarchical priors to account for variation among replicates within blocks, that is to estimate the block effects, θ_j. Specifically, we placed a normal prior on the $\theta_{\boldsymbol{j}}$ with the mean and sd estimated from the data. We placed relatively uninformative priors on these hyper-parameters, $\mu \sim$ normal(mean=0, sd=10), $\sigma \sim \gamma$ (2, 0.1).

Hamiltonian Monte Carlo was then used to draw samples from a posterior distribution. Model fitting was performed using the R interface (version 4.1.2) with stan implemented by rstan (Version 2.21.5).[39] We combined results from 4 independent chains with 10,000 iterations per chain, with each chain having a burn-in of 4000 iterations and a thinning interval of 1. In addition to estimating model parameters, we obtained the posterior for the probability of recapturing striped individuals for each treatment within each block. See Figure S1.

Approximate Bayesian Computation

We used approximate Bayesian computation (ABC) to explicitly estimate the strength of selection on color-pattern in each treatment. For this, we defined selection coefficients from relative fitness values. Specifically, we set the relative fitness of stripe to 1.0 and defined the relative fitness of green as fitness_green/fitness_stripe. We then defined the selection coefficient S as relative_fitness_stripe – relative_fitness_green (i.e., the difference in the relative fitnesses). We placed uninformed prior on S bounded by -0.9 and 0.9, with independent priors on S for high- versus low-abundance treatments. As is standard in ABC, we sampled values of S from this prior for simulations. We used the sampled values of S derived from the priors to calculate the expected proportion of recaptured striped under each treatment. We added a random effect in order to account for differences between experimental bushes. Specifically, we drew the expected proportion of striped individuals for each bush from a beta distribution with alpha = $V*\pi$ and $\beta = V * (1-\pi)$ (with $\pi = -1\ (1/(S-2))$) equal to the overall expected proportion of striped individuals for each treatment and V a scalar that affects the variance around each experimental bush). V was drawn from a uniform prior bounded by 2 and 25. We then use the derived expected proportions of striped individuals per bush in order to sample survival based on the number of recaptured individuals to estimate the expected number of striped and unstriped individuals.We ran a total of 10 million simulations using the R interface (version 4.1.2). Then we selected the 0.01% (1000) simulations with S closest to the empirical data to calculate the posterior probabilities for each treatment using a local linear correction using the *abc* package in R.[40]