

Evolution of Camouflage Drives Rapid Ecological Change in an Insect Community

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Summary

Background: Evolutionary change in individual species has been hypothesized to have far-reaching consequences for entire ecological communities [1–3], and such coupling of ecological and evolutionary dynamics (“eco-evolutionary dynamics”) has been demonstrated for a variety of systems [4–7]. However, the general importance of evolutionary dynamics for ecological dynamics remains unclear. Here, we investigate how spatial patterns of local adaptation in the stick insect *Timema cristinae*, driven by the interaction between multiple evolutionary processes, structure metapopulations, communities, and multitrophic interactions.

Results: Observations of a wild *T. cristinae* metapopulation show that locally imperfect camouflage reduces population size and that the effect of such maladaptation is comparable to the effects of more traditional ecological factors, including habitat patch size and host-plant species identity. Field manipulations of local adaptation and bird predation support the hypothesis that maladaptation reduces population size through an increase in bird predation. Furthermore, these field experiments show that maladaptation in *T. cristinae* and consequent increase in bird predation reduce the pooled abundance and species richness of the co-occurring arthropod community, and ultimately cascade to decrease herbivory on host plants. An eco-evolutionary model of the observational data demonstrates that the demographic cost of maladaptation decreases habitat patch occupancy by *T. cristinae* but enhances metapopulation-level adaptation.

Conclusions: The results demonstrate a pervasive effect of ongoing evolution in a spatial context on population and community dynamics. The eco-evolutionary model makes testable predictions about the influence of the spatial configuration of the patch network on metapopulation size and the spatial scale of adaptation.

Introduction

Historically, evolution has generally not been considered important in driving ecological patterns and processes (but see, e.g., [8, 9]). During most of the last century, the

opinion prevailed that evolution occurs too slowly to drive ecological processes, which occur rapidly in comparison [10, 11]. In the past few decades, however, numerous examples of rapid evolution have been documented in a wide range of organisms [11–13], stimulating further research into potential ecological effects of evolution [2, 3, 10, 11]. Convincing demonstrations of so-called “eco-evolutionary dynamics” are accumulating, and evolution has been shown to affect population demography [14–17], community structure [5–7], and ecosystem functioning [5, 6]. Despite this boom in eco-evolutionary studies, the present body of eco-evolutionary research generally lacks explicit consideration of spatial dynamics ([1], but see, e.g., [18]). Instead, studies either focus on ecological dynamics through time (e.g., [4, 15, 16]) or consider space implicitly (e.g., [5–7]). Additionally, a number of other fundamental issues regarding eco-evolutionary dynamics remain understudied, including the magnitude of ecological effects of evolution relative to the effects of traditional ecological factors ([3], but see [14, 19, 20]), the ecological mechanisms that mediate eco-evolutionary effects (such as predation, mutualism, and competition), and the ecological effects of fundamental evolutionary processes other than natural selection, such as gene flow and founder effects (but see [21]).

Here we address these issues by integrating the study of spatial habitat heterogeneity into eco-evolutionary dynamics. Habitat heterogeneity is a highly relevant context for studying the effects of evolution on ecology, since it is well known to have important ecological [22] and evolutionary [23] consequences. The ecological effects of habitat heterogeneity have been examined under the paradigm of source-sink dynamics [24, 25], where “source” and “sink” populations occupy high- and low-quality habitat patches, respectively. However, habitat patch quality depends not on habitat type alone, but on the interaction between habitat type and the phenotypic composition of resident populations. Hence, phenotypic evolution has the potential to structure patterns of habitat quality across the landscape, driven by spatial variation in the relative influence of evolutionary processes on local populations, such as natural selection, gene flow, founder effects, genetic drift, and mutation [26]. Here, we study spatial eco-evolutionary dynamics using a field system in speciation biology, the stick insect *Timema cristinae* [27].

Timema cristinae is a flightless, folivorous stick insect (Order: Phasmatodea) endemic to a small region (~30 km²) of mountainous chaparral habitat in the Coast Range of southern California, US, where it is the only resident species of the genus *Timema*. *Timema cristinae* is particularly abundant on the two dominant shrub species *Adenostoma fasciculatum* (Rosaceae) and *Ceanothus spinosus* (Rhamnaceae) (“*Adenostoma*” and “*Ceanothus*” hereafter). These plant species are morphologically dissimilar, especially in the shape of their leaves, with *Adenostoma* having fascicled, needle-like leaves and *Ceanothus* having broad, ovate leaves (Figures 1C and 1D). Accordingly, strong divergent natural selection from avian predators has caused the evolution of two morphologically distinct ecotypes of *T. cristinae*, each being more cryptic on

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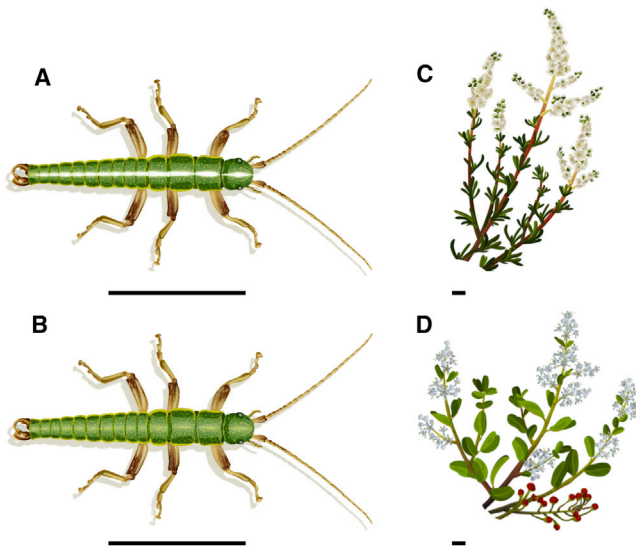


Figure 1. *Timema cristinae* Morphs and Host Plants
Striped morph (A), green morph (B), *Adenostoma fasciculatum* (C), and *Ceanothus spinosus* (D). Black bars depict approximate 1 cm scale.

one of the two plant species [28, 29]. The two *T. cristinae* ecotypes differ in a suite of morphological characters, but the most obvious difference is the presence versus absence of a highly heritable, white dorsal stripe, distinguishing the “striped” and “green” morphs (Figures 1A and 1B), which is the focus of the current study. Previous manipulative experiments, supported by the present study, have shown that striped individuals are more cryptic on *Adenostoma* than on *Ceanothus*, whereas green individuals are more cryptic on *Ceanothus* than on *Adenostoma* [30].

While natural selection works to make populations better camouflaged to their respective host plants, gene flow between *T. cristinae* populations on different hosts hinders local adaptation. Consequently, populations inhabiting regions where *Adenostoma* and *Ceanothus* are intermixed tend to be more poorly camouflaged than those existing in isolation from the alternate host species [29, 31, 32]. Thus, morphological variation in *T. cristinae* is determined by a balance between divergent selection and gene flow, resulting in marked spatial variation in the degree of local adaptation. In addition, founder events are likely to play a role in determining population-level phenotypes, especially in small, extinction-prone populations, as will be discussed below. For the purposes of this paper, “local adaptation” is defined quantitatively as the proportion of individuals in a population of the well-adapted morph (proportion green on *Ceanothus*, proportion striped on *Adenostoma*). “Maladaptation” is defined as 1 minus this value and will be primarily used throughout this paper.

The *T. cristinae* system offers an excellent opportunity to investigate the interplay between evolution and ecology in a spatial context. We address six eco-evolutionary hypotheses with this study system:

- (1) Natural selection, gene flow, and founder effects drive variation in local adaptation across landscapes, creating a spatial mosaic of local adaptation/maladaptation.
- (2) Maladaptation correlates negatively with population size (see hypothesis 3) and has an effect size comparable to

those of ecological factors such as host-plant species identity and habitat patch size.

- (3) The effect of maladaptation on population size is caused by bird predation, as birds are attracted to and/or forage more efficiently on populations of *T. cristinae* composed of poorly camouflaged individuals.
- (4) Maladaptation reduces the density and richness of arthropods cohabiting with *T. cristinae* because birds attracted to maladapted populations eat and/or scare them away.
- (5) Effects of maladaptation on non-*Timema* arthropods create a trophic cascade, decreasing levels of herbivory.
- (6) Maladaptation creates an eco-evolutionary feedback by increasing the risk of local extinction due to reduced population sizes, which has consequences for metapopulation size but also the spatial pattern of adaptation via founder effects in the course of subsequent colonizations.

If these six hypotheses are supported, there is strong evidence for habitat heterogeneity generating genuine reciprocal eco-evolutionary dynamics with consequences across multiple trophic levels. To address these hypotheses, we combine an observational field study, two manipulative field experiments, and eco-evolutionary modeling. In the observational study, we mapped the occurrence of local populations of *T. cristinae* throughout a 63 × 35 m mixed stand of the two host-plant species (*Adenostoma* n = 117, *Ceanothus* n = 69) and exhaustively searched each patch for *T. cristinae*, recording abundance and morph frequency (striped versus green). This observational study contrasts with previous field studies of *T. cristinae* by examining very small habitat patches often composed of single plant individuals, rather than large patches composed of many plant individuals. It allows explicit tests of hypotheses 1 and 2. We then use two field experiments to test hypotheses 2 through 5. In the first field experiment, we manipulated local adaptation by transplanting wild-caught *T. cristinae* of striped, green, or *Ceanothus*-adapted (see the [Supplemental Experimental Procedures](#) available online) to wild bushes of *Adenostoma*. We compare the effects of transplanting the well-adapted morph (striped) with the effects of transplanting the two types of maladapted individuals. The second experiment, conducted in the following year, complements the first by crossing the maladaptation treatment with a bird-exclusion treatment, allowing for an explicit test of hypothesis 3. Finally, we parameterize a recently developed eco-evolutionary metapopulation model [21] with the observational data to further address hypothesis 1 and use the model to infer large-scale and long-term consequences of evolutionary dynamics (hypothesis 6). The model can be used to make testable predictions about the influence of the spatial configuration of the habitat patch network on metapopulation size and the spatial scale of adaptation.

Results

Natural Selection, Gene Flow, and Founder Effects Structure Local Adaptation

We found support for the hypothesis that natural selection yields locally adapted populations. In the observational study, the green morph was significantly more common on *Ceanothus*, where it is more cryptic, and vice versa for the striped morph on *Adenostoma*. This finding agrees with prior research on *T. cristinae* [28, 32], but provides novel evidence for strong

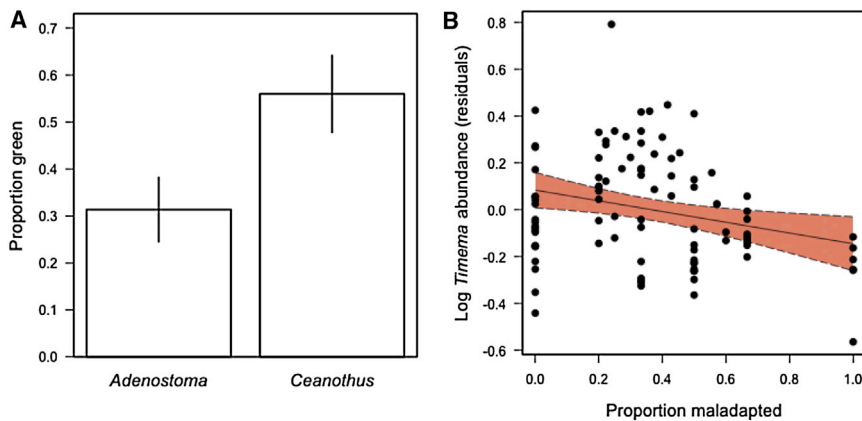


Figure 2. Metapopulation Patterns

(A) Mean frequency of the green morph on the two host plant species ($n = 100$ patches). Error bars depict 95% confidence intervals. The difference in morph frequency between the host plant species is highly significant (Table 1, “Phenotype”; $p < 0.001$).

(B) Residual *Timema* abundance from multiple regression model not including the effect of maladaptation versus the proportion of maladapted individuals in each local population. The solid line is the best fit, and the pink band within dotted lines depicts 95% confidence intervals. The effect of maladaptation is highly significant (Table 1, “Population Size”; $p = 0.005$).

selection at the very small scale of host-plant individuals (Figure 2A and Table 1, “Phenotype”).

Local morph frequencies were not significantly affected by the connectivity of the populations to the surrounding other populations or by morph frequencies in the surrounding populations (Table 1, “Phenotype,” and the Experimental Procedures). These results suggest that dispersal and gene flow are not so high that they would significantly affect local population sizes and morph frequencies at this very small scale. In other words, *T. cristinae* populations on individual plant patches represent relatively independent local populations (see the Experimental Procedures for tests of spatial autocorrelation).

We fitted an eco-evolutionary model (Experimental Procedures) to the data from the observational study. The data consist of a single snapshot of spatial data, shown in Figure 3C, and thus provide insufficient information to estimate the values of all model parameters independently. Thus, we fixed several parameters with independent data (the spatial range of dispersal) or set them at values that are consistent with the experimental data (strength of local selection; see below and the Experimental Procedures). This left us with two parameters to estimate from the observational data in Figure 3C: rates of dispersal and gene flow. The model parameterized in this way fitted well the empirical patterns (Figures 3B–3D and S1A–S1C). In particular, compare Figure 3C, which gives the empirical morph frequency in the 48 local populations with at least four *Timema* sampled, with Figure 3D, which shows the model-predicted pattern for an identical sample of individuals (sampled from the model-predicted equilibrium morph distribution shown in Figure 3B; Experimental Procedures). Hence, we conclude that spatial variation in morph frequency is consistent with the eco-evolutionary dynamics in the model, including strong local selection on morph frequency and short spatial range of dispersal and gene flow. Below, we use the model to analyze eco-evolutionary dynamics in this system and to make new testable predictions.

Maladaptation Decreases Population Size

We found strong support for this hypothesis. In the observational study, we found a significant negative correlation between the proportion of maladapted individuals in a patch and population size (Table 1, “Population Size,” and Figure 2B). In addition, we found that population size was on average higher on *Ceanothus* than on *Adenostoma* and that it increased with the size of the patch (Table 1, “Population Size”). The effect size (partial adjusted R^2) of maladaptation

on population size ($R^2 = 0.071$) was comparable to that of patch size ($R^2 = 0.138$) and host-plant species ($R^2 = 0.032$), suggesting that evolutionary dynamics are of similar importance to traditional ecological factors in determining population size in this case.

Both field experiments further supported the hypothesis that maladaptation reduces population size, showing significantly lower *T. cristinae* abundance in maladapted treatments relative to well-adapted treatments (Table 2 and Figures 4A and 4E), establishing a causal association between maladaptation and population size. The effect size of maladaptation was large, as judged by adjusted R^2 values for the treatment effect in both experiments (experiment 1, 0.35; experiment 2, 0.12; see the Supplemental Experimental Procedures for details). Finally, though we do not have empirical data for this, it is very likely that the risk of population extinction increases with decreasing population size, and hence maladaptation is expected to increase the risk of local extinction. This is the key assumption of the model whose results are shown in Figure 3 (Experimental Procedures).

Bird Predation Drives the Effect of Local Adaptation on Population Size

In the bird-exclusion experiment, we found that the maladapted treatment harbored significantly lower *T. cristinae* abundance than did the well-adapted treatment when birds were allowed to forage, but not when birds were excluded (Figure 4E and Table 2, “Experiment 2”). The interaction between treatments was statistically significant (Table 2, “Experiment 2”), supporting the hypothesis that bird predation mediates the effects of maladaptation on *T. cristinae* population size.

Maladaptation Decreases the Abundance and Diversity of Arthropods

We found experimental evidence that local maladaptation in *T. cristinae* populations reduces both the abundance and diversity of co-occurring, non-*Timema* arthropods (Table 2, “Combined Probabilities”). In the first manipulative experiment, we found that the pooled abundance of non-*Timema* arthropods and their species richness were significantly lower in the maladapted treatments than in the well-adapted (striped) treatment (abundance $R^2 = 0.12$, richness $R^2 = 0.28$; Figures 4B and 4C and Table 2, “Experiment 1”). Effects on species richness may be due to the exclusion of rare species by predators, a phenomenon known to occur in terrestrial systems [33, 34].

The bird-exclusion experiment corroborates these findings and further supports bird predation as the mechanism driving

Table 1. Summary Statistics for Multiple Regression Models Testing the Factors Associated with Variation in Mean Phenotype and Local Population Size

Variable	df	Adjusted R^2	t	p
Phenotype				
Host species	1, 95	0.136	4.01	<0.001
$q^{\text{imm-A}}$	1, 95	-0.010	-0.25	0.801
$q^{\text{imm-C}}$	1, 95	-0.001	-0.95	0.349
Connectivity	1, 95	-0.009	0.20	0.842
Population Size				
Maladaptation	1, 95	0.071	-2.88	0.005
Patch size	1, 95	0.138	4.04	<0.001
Host species	1, 95	0.032	2.03	0.046
Connectivity	1, 95	0.010	1.41	0.162

The two dependent variables are the proportion of the green morph in 100 plant patches (“Phenotype”) and log *T. cristinae* population size in those same patches (“Population Size”). $q^{\text{imm-A}}$ and $q^{\text{imm-C}}$ are the proportion of green immigrants to a patch from surrounding *Adenostoma* and *Ceanothus* patches, respectively. Significant effects at $\alpha = 0.05$ are indicated with bold, italicized text.

the effects of maladaptation on both arthropod abundance and diversity. There was significantly lower abundance and species richness of non-*Timema* arthropods in the maladapted treatment than in the well-adapted treatment when birds were allowed to forage (abundance $R^2 = 0.32$, richness $R^2 = 0.41$), but not when birds were excluded (Figures 4F and 4G and Table 2, “Experiment 2”). The statistical interaction between the bird-exclusion treatment and the adaptation treatment was marginally significant for arthropod abundance and significant for arthropod diversity (Table 2, “Experiment 2”).

Maladaptation Decreases Herbivory from Sap-Feeding Arthropods

Consistent with the effects of local adaptation on non-*Timema* arthropod abundance, we found that maladaptation in *T. cristinae* reduces the level of herbivory from non-*Timema* arthropods (Table 2, “Combined Probabilities”). In the first manipulative experiment, the proportion of leaves showing damage from sap-feeding herbivores (e.g., plant hoppers) was lower on maladapted treatments than on well-adapted treatments ($R^2 = 0.54$; Table 2, “Experiment 1,” and Figure 4D).

In the bird-exclusion experiment, there was significantly lower herbivory by sap-feeding arthropods in the maladapted treatment than in the well-adapted treatment when birds were allowed to forage ($R^2 = 0.11$), but not when birds were excluded (Figure 4H and Table 2, “Experiment 2”). The statistical interaction between the bird-exclusion treatment and the adaptation treatment was marginally significant (Table 2, “Experiment 2”).

Local Maladaptation Generates an Eco-Evolutionary Feedback and Has Network-Level Consequences for Habitat Patch Occupancy and the Spatial Scale of Adaptation

The key eco-evolutionary feature of the model (Experimental Procedures) is increased risk of local extinction due to maladaptation, which is consistent with the empirically observed reduction in population size due to maladaptation (Figure 2B). Here, we compare the predictions of the full eco-evolutionary model explicitly including the demographic cost of maladaptation with a reduced model in which the demographic cost of

maladaptation is set to zero and thus this eco-evolutionary feedback is cut off (see the Experimental Procedures). This comparison shows that the cost of local maladaptation results in reduced probability of habitat patch occupancy (Figure 3F), due to increased risk of local extinction of maladapted populations. Thus, local maladaptation reduces the overall size of the metapopulation.

Furthermore, the cost of maladaptation generates an eco-evolutionary feedback affecting the spatial scale of adaptation. Specifically, our model demonstrates a smoothing of spatial variation in morph frequency, reducing strict patch-specific local adaptation and promoting adaptation across a larger part of the patch network. In the present case, the metapopulation in the northern part of the network, dominated by *Ceanothus*, becomes increasingly dominated by the green morph, while the frequency of the striped morph becomes elevated in the southern, *Adenostoma*-dominated part of the network. Figures 3E and 3G show the difference in morph frequency between the full model with the eco-evolutionary feedback and the reduced model without the feedback (Figure 3E shows the patch-specific change in morph frequency on the map; Figure 3G compares the patch-specific morph frequencies of the two models). The broader conclusion emerging from these results is that the effects of evolutionary change on ecology (here population size) subsequently feed back to influence the evolutionary trajectory of the species (the spatial scale and extent of host-plant adaptation).

Discussion

Research into eco-evolutionary dynamics has primarily examined two scenarios. First, recent studies demonstrate how evolutionary change may influence ecological dynamics. For instance, body-size evolution in wild sheep influences their population dynamics [16], body-shape evolution in stickleback influences the community structure of their prey [6], and adaptation to predation regimes in guppies alters the relative abundance of algae and invertebrates and modifies invertebrate community structure [5]. Second, laboratory studies on predator-prey dynamics have exemplified eco-evolutionary feedbacks, which involve the reciprocal and potentially ongoing influence of ecological dynamics on evolutionary change and vice versa [4, 35]. The empirical study of such interactions is facilitated by the common occurrence of evolution happening on contemporary time scales, well within the purview of experimental studies. Evolutionary ecologists have recently been particularly interested in these scenarios, wherein rapid evolutionary change influences ecology, since they are understudied relative to research into evolution by natural selection, which is typically based on the ecology of the species, and for which a vast literature exists [3, 12, 13, 36].

Despite many excellent empirical demonstrations of eco-evolutionary dynamics, direct comparisons of how ecological versus evolutionary factors influence the dynamics of populations, communities, and ecosystems are needed to gauge just how important rapid evolution is as an ecological force [3]. Only a few studies [14, 20] have done such a comparison, and they found that the ecological and evolutionary factors can be comparable in magnitude. Our results strongly support these studies, as in our case the effect of maladaptation on population size was roughly comparable with the effects of habitat patch size and host-plant species identity. Furthermore, we report R^2 values for the effect of maladaptation

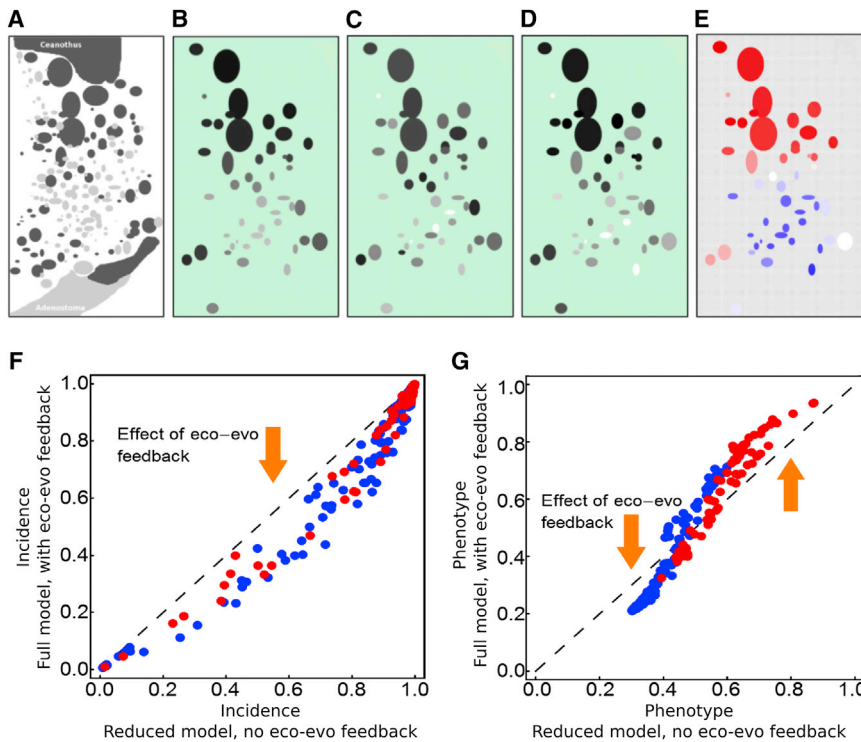


Figure 3. Fit of the Eco-Evolutionary Metapopulation Model to Observational Data

(A) A map of the patch network within the 63 by 35 m study area (dark patches are *Ceanothus*, light patches are *Adenostoma* plant individuals/patches).

(B) The model-predicted long-term equilibrium frequency of the green morph in local populations, with darker shading representing higher frequency of the green morph, which is well adapted to *Ceanothus*.

(C) The empirically observed frequency of the green morph.

(D) The frequency of the green morph in a sample corresponding to the empirical sample in (C) and drawn from the model-predicted morph frequencies in (B).

(A–D) Note that the model-predicted (D) and the observed (C) frequency of the green morph show a very similar spatial pattern across the patch network. Figure S1 shows a further analysis of the fit of the model to the observed phenotypes.

(E–G) A comparison between the predictions of the full model including the eco-evolutionary feedback (of maladaptation increasing the extinction risk) and a reduced model in which this feedback has been set to zero. The predicted probability (incidence) of patch occupancy is reduced by the feedback (F), while the frequency of the green (*Ceanothus*-adapted) morph is elevated in the *Ceanothus*-dominated part of the network and vice versa for the *Adenostoma*-

dominated part of the network (G). Each point is one plant patch, red indicating *Ceanothus* and blue *Adenostoma*. (E) shows the difference in the predicted frequency of the green morph in local populations between the two models on a map, with red and blue denoting higher and lower proportions of the green morph in the full model, respectively.

(A), (F), and (G) show all host plant patches ($n = 186$), whereas (B)–(E) show the patches with at least four *Timema* sampled ($n = 48$). See also Figure S1.

treatments in both experiments, which are generally large. These effect sizes are upper estimates in the sense that the maladaptation treatments represent maximally maladapted populations, though we note that equally severe maladaptation does occur in nature, where small patches of one host species are embedded within or near a large network of the alternate host ([31]; see also Figures 3A and 3C).

Evolutionary change may have ecological consequences that extend beyond the focal species. In the present case, we identified an indirect interaction akin to apparent competition, where in theory an increase in the abundance of either of two prey species causes a reduction in the abundance of the second prey species due to interactions with a shared predator [37]. Here, the phenotypic composition of *T. cristinae* populations influenced the rate of predation on non-*Timema* prey species, reducing their abundance (Figures 4B and 4F) and even excluding some species (Figures 4C and 4G). This indirect effect is trait mediated (sensu [38, 39]), because *T. cristinae* phenotypes influence other arthropods through shared effects of bird behavior, but also in the sense that the evolution of *T. cristinae* phenotypes, rather than changes in *T. cristinae* abundance, drives the effects. We note here, though, that our experiments were not designed to address the relative roles of a populations' phenotypic composition and its total abundance, both of which are expected to affect predation rate. We nevertheless consider this an intriguing avenue for future study. Trophic cascades are another well-known indirect interaction, are well documented in both aquatic and terrestrial habitats, and can have major effects on populations, communities, and

ecosystems [40, 41]. However, the effects of evolutionary dynamics on the strength of trophic cascades have remained little studied ([5, 6], but see [42]). Here, we add to this literature a strong example of an evolution-mediated trophic cascade (Figures 4D and 4H).

The theory of island biogeography [43] is another classic paradigm in community and metapopulation ecology to which our work is related. Under this paradigm, habitat isolation is predicted to decrease species richness via reduced colonization [43, 44]. In our study system, habitat patch isolation could have the opposite influence on species richness, because local adaptation is promoted by isolation [29, 32] and would here increase arthropod diversity (Table 2 and Figures 4C and 4G). Thus, the effect of isolation on local adaptation may reduce, nullify, or even invert the diversity effects of isolation stemming from dispersal and colonization dynamics. Though this specific scenario may not apply to many systems, we suggest that where evolutionary dynamics occur in species that are important members of a community, evolutionary effects may lead to deviations from traditionally predicted patterns of biodiversity.

The significance of spatial demographic dynamics in a heterogeneous environment for driving eco-evolutionary dynamics further reveals an important insight to the temporal stability of evolutionary effects on ecology. Often, the effect of evolutionary change on ecological change will become weaker with increasing adaptation and the consequent deceleration of evolutionary change. In such situations, eco-evolutionary dynamics would be important for ecological change only during the transient period of adaptation (but see [10]).

Table 2. Effect of Maladaptation in Two Manipulative Experiments for Four Community Variables

	<i>T. cristinae</i> Abundance		Arthropod Abundance		Arthropod Richness		Herbivory	
	z	p	z	p	z	p	z	p
Experiment 1								
Treatment	-5.34	<0.001	-3.01	0.003	-2.19	0.029	-3.48	<0.001
Experiment 2 ^a								
Open	-1.97	0.025	-2.47	0.007	-2.15	0.016	-2.07	0.019
Exclusion	0.71	0.710	-0.85	0.199	0.00	0.500	0.04	0.514
Interaction	-1.78	0.038	-1.43	0.076	-1.73	0.041	-1.44	0.076
Combined Probabilities ^a								
Treatment	-5.68	<0.001	-3.24	< 0.001	-2.39	0.008	-3.55	<0.001

For experiment 2, “open” and “exclusion” rows report statistics for the effect of maladaptation treatment when birds were allowed and prevented from foraging, respectively. The “interaction” row reports statistics for interaction between adaptation and bird-exclusion treatments. Combined probabilities of experiments 1 and 2 (see the [Supplemental Experimental Procedures](#)) are also shown. Bold, italicized text indicates significance at $\alpha = 0.05$.

^ap values are one-tailed.

The present study exemplifies a mechanism that may sustain eco-evolutionary dynamics over long periods. In this scenario, rapid evolutionary dynamics do not require systematic temporal change in the environment. Instead, coupling of demographic and evolutionary dynamics is generated by interactions between local populations inhabiting dissimilar habitat patches, whereby ongoing natural selection and gene flow have opposing effects on adaptation, with consequences for local ecological dynamics. Local extinctions and recolonizations generate further variation in the phenotypic composition of populations via founder effects. Situations in which natural selection, gene flow, and founder events create spatial variation in the phenotypic composition of populations can be expected to be common in environments with small-scale heterogeneity in environmental conditions (e.g., [45–47]), and such scenarios are good candidates for sustained eco-evolutionary dynamics [48]. One trait for which convincing evidence of eco-evolutionary dynamics has been reported is dispersal rate, which directly influences demography and local adaptation in heterogeneous environments [18].

The eco-evolutionary metapopulation model parameterized with empirical data makes testable predictions about the long-term and large-scale consequences of local maladaptation in the habitat patch network that we have studied here (Figures 3E and 3G). However, model predictions can be equally generated for other patch networks with different relative abundances and spatial configuration of the two host-plant species. Testing such predictions with additional empirical data would constitute a strong test of the model and the general importance of eco-evolutionary dynamics in the *T. cristinae* system.

In summary, using observational and experimental field evidence, we demonstrate that variation in adaptive camouflage, driven by a dynamic balance between natural selection, gene flow, and founder effects, influences spatial patterns of population demography, arthropod community structure, and rates of herbivory by affecting the strength of bird predation. Using mathematical modeling, we examine the role of an important eco-evolutionary feedback, namely maladaptation increasing the rate of local extinction, in influencing ecological and evolutionary metapopulation dynamics. In concert, these results point to the general importance and pervasive influence of ongoing evolution on population and community dynamics.

Experimental Procedures

Study Sites

Empirical work was carried out within a 1 km² area of chaparral shrub-land in the Santa Ynez mountain range of coastal southern California, ~14 km NNW of Santa Barbara (Figure S3A).

Metapopulation Observations

Between April 1 and 5, 2011, we measured *T. cristinae* local population sizes and morph frequencies throughout a network of *Adenostoma* (n = 117) and *Ceanothus* (n = 69) habitat patches (Figure 3A) using sweep nets. Our goal was to sample all individuals in each patch, and hence sampling time was proportional to patch size. Of 186 total patches, 50 yielded zero individuals. One of us (P.N.) phenotyped all individuals, separating them into four morphs: green unstriped (“green” hereafter), green striped (“striped” hereafter), gray, and red. Separation of individuals into these categories is repeatable [32, 49, 50].

We divided the entire area into 7 × 7 m squares, and used a tape measure to map the location and size of each habitat patch. The rectangular-solid volume (L × W × H) of each patch was also recorded. Individual plants of the same species were considered part of a single patch if the distance between them was less than 30 cm. Maladaptation was calculated for *Adenostoma* patches as the proportion of individuals of the green morph (no. green / [no. green + no. striped]) and as the proportion of the striped morph for *Ceanothus* (see the [Supplemental Experimental Procedures](#) for consideration of red and gray morphs).

We used multiple regression to model the log-transformed total number of *T. cristinae* sampled and morph frequency in each patch as explained by maladaptation, log-transformed patch size, patch connectivity (C; see the [Supplemental Experimental Procedures](#)), q^{imm}_{-A} , q^{imm}_{-C} , and host species (Table 1). q^{imm}_A and q^{imm}_C denote the expected proportion of the green morph among immigrants to a patch from the surrounding *Adenostoma* and *Ceanothus* patches, respectively (see the [Supplemental Experimental Procedures](#)). In these analyses, we included patches with more than one individual sampled (n = 100). Analysis was performed using the base package for R statistical freeware [51]. We tested for spatial autocorrelation by comparing these full models (Table 1) with and without spatial structure using the *gstat* [52] and *nlme* [53] packages in R. The inclusion of exponential, Gaussian, rational quadratic, and spherical correlation in both models always increased Akaike information criterion (AIC) by at least four, indicating lack of spatial correlation structure.

Eco-Evolutionary Model

For a detailed description of the model, see [21]. The model has seven parameters: the strength of local selection, γ ; the amount of additive genetic variance, σ^2 ; the spatial range of dispersal, α ; the rate of dispersal, c ; a parameter scaling colonization in relation to dispersal (gene flow), ρ ; and the average and variance of local population growth rate, r_1 and v , respectively. Habitat patch area affects extinction risk and the contribution of the patch to dispersal; patch area was calculated as square root of the product of width and length.

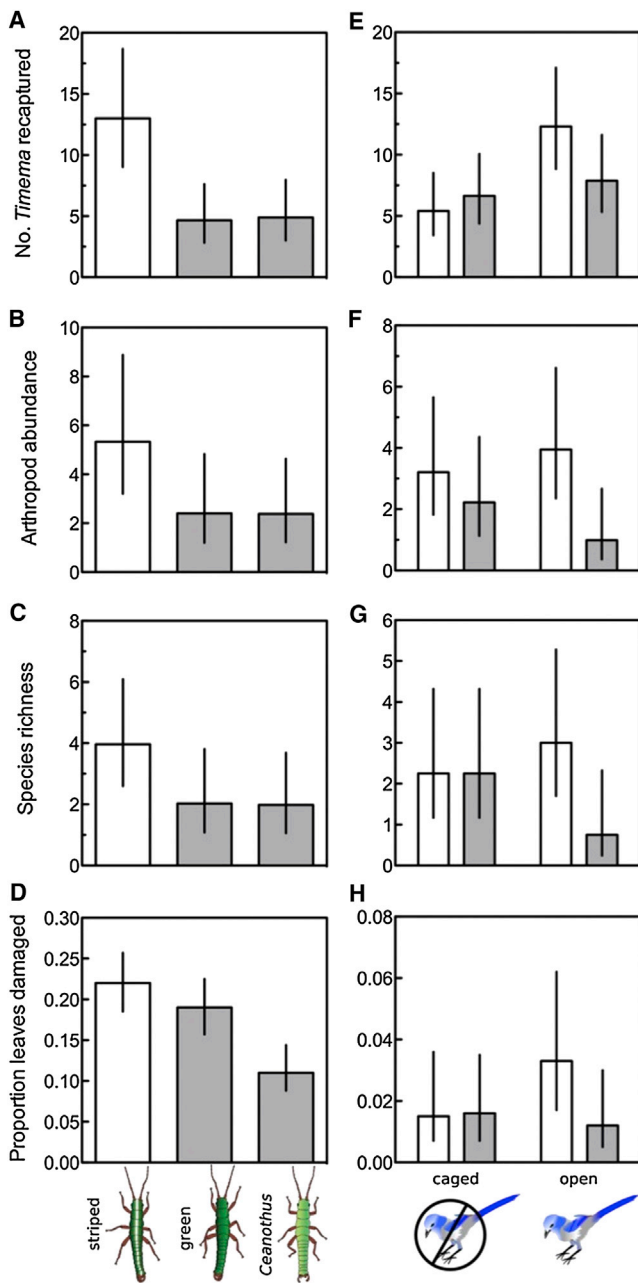


Figure 4. Population and Community Traits across the Treatments in the Manipulative Field Experiments

(A–D) Results of the first maladaptation experiment. Drawings of *T. cristinae* (courtesy of C. Brown) depict typical color of individuals for striped, green and *Ceanothus* treatments, from left to right.

(E–H) Results of the second experiment that included bird exclusion. (A and E) *T. cristinae* abundance (number of individuals recaptured). (B and F) Total non-*Timema* arthropod abundance. (C and G) Arthropod species richness (number of morphospecies). (D and H) The proportion of leaves sampled with sap-feeding herbivore damage.

White bars, striped treatment; gray bars, maladapted treatment(s). Bars depict marginal means from GLMMs with block as a random factor (A–H) and the logarithm of plant volume as a fixed covariate (A–C). Error bars depict 95% confidence intervals. Statistical tests are reported in Table 2. See also Figure S2.

We fitted the model using the observational data from local populations with $n \geq 4$ individuals recorded ($n = 48$ local populations), though model predictions were generated for all 186 patches. We fitted the deterministic approximation of long-term equilibrium to the data [21]; hence, we assume that the empirical data approximate a long-term steady state. As the empirical data consist of only a single snapshot of spatial data, we cannot estimate all the model parameters with the data available, and hence we fixed the following parameters to plausible values based on the observational and experimental results. We assumed that $\gamma = 5$ and $\sigma^2 = 1$, to reflect the experimental result of rapid change in morph frequency after experimental perturbation (Figure 4) [29]. As we cannot estimate the rate of population turnover with a snapshot of data, we set the local extinction rate at a fixed value for each patch area by assuming that the average local growth rate (r_i) and its variance (v) both equal 1 [21]. The spatial range of dispersal was set at $\alpha = 0.5$, which gives an average dispersal distance of 2 m, consistent with previous results [54]. Making these assumptions leaves two parameters to be estimated: the dispersal rate parameter (c) and the gene flow parameter (ρ). We estimated the values of c and ρ by evaluating all possible combinations. The sum of the predicted patch incidences was constrained to match the number of occupied patches, and we thus fitted the model to the set of mean local phenotypes conditional on the total metapopulation size being close to that observed. The fitting criterion was the sum of absolute errors between the predicted and observed mean phenotypes in the 48 local populations. A sensitivity analysis is described in the Supplemental Information (Figure S1). The average incidence of patch occupancy is most sensitive to the rate and average distance of dispersal, while the mean phenotype is most sensitive to dispersal distance and the amount of genetic variance.

To analyze the effect of maladaptation on demographic dynamics, we compared the incidences of patch occupancy and the mean phenotypes predicted by two models. The full model that was fitted to the data (Figure 3) includes the eco-evolutionary feedback of increased extinction risk with increasing local maladaptation. In the reduced model this feedback was set to zero, that is, local extinction was assumed to be independent of maladaptation, by setting $\gamma = 0$ in equation 6 in [21], and keeping all other parameters at the same values as in the full model.

Maladaptation Manipulation

On March 17, 2011, we transplanted 100 *T. cristinae* individuals to each of 15 bushes of *Adenostoma*, divided between three maladaptation treatments in five spatially replicated blocks (Figures S2A and S2B). In the striped-morph treatment, we transplanted individuals possessing a clear dorsal stripe (Figure 1A), collected from a polymorphic population inhabiting *Adenostoma* (FHA; location: N34.518, W-119.800 DD) between March 14 and 16, 2011. In the green-morph treatment, we transplanted individuals lacking a dorsal stripe collected from the same population (FHA; Figure 1B). In the *Ceanothus* treatment, we transplanted individuals from a suite of populations inhabiting *Ceanothus*, all located near site R12C (N34.515, W-120.071 DD). See the Supplemental Information for consideration of morph frequencies at FHA and R12C and rearing conditions for collected individuals.

On April 12, 2011, we sampled all experimental bushes by hitting all branches with a stick and collecting fallen individuals into a sweep net. We collected all arthropods and stored them in 70% ethanol (EtOH) for future identification. On April 16, 2011, we collected one haphazardly chosen, 10-cm-long branch tip from each experimental bush and stored them in 70% EtOH. At the same time, we measured the rectangular-solid volume of each bush ($L \times W \times H$). One of us (T.E.F.) separated all arthropods into morphospecies using a reference collection created in 2010 and measured their body length from the front of the head or cephalothorax to the rear of the abdomen. We scored herbivory for each plant by removing the 100 most basal leaves from branch clippings and determining under a dissecting microscope whether leaves were damaged or undamaged by piercing/sucking herbivores, as indicated by a small hole and ring of necrotic tissue. One laboratory assistant performed all herbivory scoring.

We used generalized linear mixed models (GLMMs) with Laplace approximation and block ($n = 5$) as a random factor to analyze the data. For analyses of *T. cristinae* abundance, arthropod abundance, and arthropod species richness, we used a Poisson error distribution with a log link function. Arthropod abundance was calculated as the total number of individuals with body length greater than 5 mm, and arthropod species richness was calculated as the number of species for which at least one individual longer than 5 mm was present. We included only individuals greater than 5 mm to allow for accurate morphospecies identification. Furthermore, the hypothesized mechanism for the observed effects in the experiment

was bird predation, and bird predators are known to forage more heavily on large prey items [55, 56] (see the bird-exclusion experiment below). The statistical models included treatment and the natural logarithm of plant volume as fixed factors. Herbivory was analyzed using a logit link function (logistic regression) with treatment as a fixed factor. We selected GLMMs over generalized linear models (GLMs) by comparing models with and without block as a random factor using AIC minimization.

Bird Exclusion

On April 19, 2012, we transplanted 38 individuals to each of 16 bushes of *Adenostoma* divided between four experimental treatments and four spatially replicated blocks (Figures S2A, S2C, and S2D). The four treatments were constructed using a 2 × 2 factorial design, crossing a maladaptation treatment with a bird-exclusion treatment. For the maladaptation treatment, we transplanted either striped or green individuals collected between April 16 and 18, 2012, from FHA (see the Supplemental Experimental Procedures). For the bird-exclusion treatment, we enclosed half of the bushes in each block (one striped and one green) with cages constructed of chicken wire. Prior to the release of *Timema*, all bushes were trimmed into ~0.9 m³ cubes to reduce variability in *T. cristinae* density across bushes.

On May 4, 2012, all bushes were sampled as in the maladaptation experiment described above. The bushes were resampled on May 5, 2012 to increase the efficiency of the sampling. Insects and haphazardly chosen 10 cm branch tips were stored in 70% EtOH. Branch tips were taken only if they were not damaged by bush trimming at the start of the experiment. All arthropods were identified to morphospecies and their length measured by T.E.F. Herbivory was scored as present versus absent for the 100 most basal leaves on the branch tips as in the previous experiment described above.

Timema cristinae abundance, non-*Timema* arthropod abundance (>5 mm in length), and arthropod species richness were analyzed using GLMMs with a log link function (Poisson regression) and block as a random factor. Herbivory was analyzed using a GLMM with logit link function (logistic regression) and block as a random factor. We selected GLMMs over GLMs as described in the maladaptation experiment above, and furthermore selected models containing both treatments (maladaptation and bird-exclusion) and their interaction by the same method. We examined the effect of maladaptation treatment in the open and closed treatments individually by dummy coding.

Accession Numbers

The Dryad DOI for the data reported in the paper is 10.5061/dryad.s90t6, accessible at <http://doi.org/10.5061/dryad.s90t6>.

Supplemental Information

Supplemental Information includes Supplemental Results, Supplemental Experimental Procedures and two figures and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2013.07.067>.

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References

- Urban, M.C., Leibold, M.A., Amarasekare, P., De Meester, L., Gomulkiewicz, R., Hochberg, M.E., Klausmeier, C.A., Loeuille, N., de Mazancourt, C., Norberg, J., et al. (2008). The evolutionary ecology of metacommunities. *Trends Ecol. Evol.* 23, 311–317.
- Pelletier, F., Garant, D., and Hendry, A.P. (2009). Eco-evolutionary dynamics. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 1483–1489.
- Schoener, T.W. (2011). The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science* 331, 426–429.
- Yoshida, T., Jones, L.E., Ellner, S.P., Fussmann, G.F., and Hairston, N.G., Jr. (2003). Rapid evolution drives ecological dynamics in a predator-prey system. *Nature* 424, 303–306.
- Bassar, R.D., Marshall, M.C., López-Sepulcre, A., Zandonà, E., Auer, S.K., Travis, J., Pringle, C.M., Flecker, A.S., Thomas, S.A., Fraser, D.F., and Reznick, D.N. (2010). Local adaptation in Trinidadian guppies alters ecosystem processes. *Proc. Natl. Acad. Sci. USA* 107, 3616–3621.
- Harmon, L.J., Matthews, B., Des Roches, S., Chase, J.M., Shurin, J.B., and Schluter, D. (2009). Evolutionary diversification in stickleback affects ecosystem functioning. *Nature* 458, 1167–1170.
- Johnson, M.T.J., Vellend, M., and Stinchcombe, J.R. (2009). Evolution in plant populations as a driver of ecological changes in arthropod communities. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 1593–1605.
- Chitty, D. (1960). Population processes in the vole and their relevance to general theory. *Can. J. Zool.* 38, 99–113.
- Pimentel, D. (1968). Population regulation and genetic feedback. Evolution provides foundation for control of herbivore, parasite, and predator numbers in nature. *Science* 159, 1432–1437.
- Hairston, N.G., Ellner, S.P., Geber, M.A., Yoshida, T., and Fox, J.A. (2005). Rapid evolution and the convergence of ecological and evolutionary time. *Ecol. Lett.* 8, 1114–1127.
- Thompson, J.N. (1998). Rapid evolution as an ecological process. *Trends Ecol. Evol.* 13, 329–332.
- Hendry, A.P., and Kinnison, M.T. (1999). The pace of modern life: Measuring rates of contemporary microevolution. *Evolution* 53, 1637–1653.
- Reznick, D.N., and Ghalambor, C.K. (2001). The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* 112–113, 183–198.
- Ezard, T.H.G., Côté, S.D., and Pelletier, F. (2009). Eco-evolutionary dynamics: disentangling phenotypic, environmental and population fluctuations. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 1491–1498.
- Turcotte, M.M., Reznick, D.N., and Hare, J.D. (2011). The impact of rapid evolution on population dynamics in the wild: experimental test of eco-evolutionary dynamics. *Ecol. Lett.* 14, 1084–1092.
- Pelletier, F., Clutton-Brock, T., Pemberton, J., Tuljapurkar, S., and Coulson, T. (2007). The evolutionary demography of ecological change: linking trait variation and population growth. *Science* 315, 1571–1574.
- Ozgul, A., Childs, D.Z., Oli, M.K., Armitage, K.B., Blumstein, D.T., Olson, L.E., Tuljapurkar, S., and Coulson, T. (2010). Coupled dynamics of body mass and population growth in response to environmental change. *Nature* 466, 482–485.
- Hanski, I., and Mononen, T. (2011). Eco-evolutionary dynamics of dispersal in spatially heterogeneous environments. *Ecol. Lett.* 14, 1025–1034.
- Ellner, S.P., Geber, M.A., and Hairston, N.G., Jr. (2011). Does rapid evolution matter? Measuring the rate of contemporary evolution and its impacts on ecological dynamics. *Ecol. Lett.* 14, 603–614.
- Palkovacs, E.P., Marshall, M.C., Lamphere, B.A., Lynch, B.R., Weese, D.J., Fraser, D.F., Reznick, D.N., Pringle, C.M., and Kinnison, M.T. (2009). Experimental evaluation of evolution and coevolution as agents of ecosystem change in Trinidadian streams. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 1617–1628.
- Hanski, I., Mononen, T., and Ovaskainen, O. (2011). Eco-evolutionary metapopulation dynamics and the spatial scale of adaptation. *Am. Nat.* 177, 29–43.
- Levin, S.A. (1992). The problem of pattern and scale in ecology: The Robert H. MacArthur Award Lecture. *Ecology* 73, 1943–1967.
- Kawecki, T.J., and Ebert, D. (2004). Conceptual issues in local adaptation. *Ecol. Lett.* 7, 1225–1241.
- Kawecki, T.J. (2004). Ecological and evolutionary consequences of source-sink population dynamics. In *Ecology, Genetics, and Evolution of Metapopulations*, I. Hanski and O.E. Gaggiotti, eds. (Amsterdam: Elsevier Academic Press), pp. 387–414.
- Dias, P.C. (1996). Sources and sinks in population biology. *Trends Ecol. Evol.* 11, 326–330.
- Crespi, B.J. (2000). The evolution of maladaptation. *Heredity (Edinb)* 84, 623–629.
- Nosil, P. (2007). Divergent host plant adaptation and reproductive isolation between ecotypes of *Timema cristinae* walking sticks. *Am. Nat.* 169, 151–162.

28. Nosil, P., and Crespi, B.J. (2006). Experimental evidence that predation promotes divergence in adaptive radiation. *Proc. Natl. Acad. Sci. USA* *103*, 9090–9095.
29. Nosil, P. (2009). Adaptive population divergence in cryptic color-pattern following a reduction in gene flow. *Evolution* *63*, 1902–1912.
30. Nosil, P., Sandoval, C.P., and Crespi, B.J. (2006). The evolution of host preference in allopatric vs. parapatric populations of *Timema cristinae* walking-sticks. *J. Evol. Biol.* *19*, 929–942.
31. Bolnick, D.I., and Nosil, P. (2007). Natural selection in populations subject to a migration load. *Evolution* *61*, 2229–2243.
32. Sandoval, C.P. (1994). The effects of the relative geographic scales of gene flow and selection on morph frequencies in the walking-stick *Timema cristinae*. *Evolution* *48*, 1866–1879.
33. Spiller, D.A., and Schoener, T.W. (1998). Lizards reduce spider species richness by excluding rare species. *Ecology* *79*, 503–516.
34. Schoener, T.W., and Spiller, D.A. (1996). Devastation of prey diversity by experimentally introduced predators in the field. *Nature* *381*, 691–694.
35. Becks, L., Ellner, S.P., Jones, L.E., and Hairston, N.G., Jr. (2010). Reduction of adaptive genetic diversity radically alters eco-evolutionary community dynamics. *Ecol. Lett.* *13*, 989–997.
36. Endler, J.A. (1986). *Natural Selection in the Wild* (Princeton: Princeton University Press).
37. Holt, R.D. (1977). Predation, apparent competition, and the structure of prey communities. *Theor. Popul. Biol.* *12*, 197–29.
38. Abrams, P.A. (2007). Defining and measuring the impact of dynamic traits on interspecific interactions. *Ecology* *88*, 2555–2562.
39. Abrams, P.A. (1995). Implications of dynamically variable traits for identifying, classifying, and measuring direct and indirect effects in ecological communities. *Am. Nat.* *146*, 112–134.
40. Terborgh, J., and Estes, J.A., eds. (2010). *Trophic Cascades: Predators, Prey, and the Changing Dynamics of Nature* (Washington: Island Press).
41. Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B.C., et al. (2011). Trophic downgrading of planet Earth. *Science* *333*, 301–306.
42. Bassar, R.D., Ferriere, R., López-Sepulcre, A., Marshall, M.C., Travis, J., Pringle, C.M., and Reznick, D.N. (2012). Direct and indirect ecosystem effects of evolutionary adaptation in the Trinidadian guppy (*Poecilia reticulata*). *Am. Nat.* *180*, 167–185.
43. MacArthur, R.H., and Wilson, E.O. (1967). *The Theory of Island Biogeography* (Princeton: Princeton University Press).
44. Brown, J.H., and Kodric-Brown, A. (1977). Turnover rates in insular biogeography: Effect of immigration on extinction. *Ecology* *58*, 445–449.
45. Dhondt, A., Adriaensen, F., Matthysen, E., and Kempenaers, B. (1990). Nonadaptive clutch sizes in tits. *Nature* *348*, 723–725.
46. Riechert, S. (1993). The evolution of behavioral phenotypes - Lessons learned from divergent spider populations. *Advances in the Study of Behavior* *22*, 103–134.
47. Tack, A.J.M., and Roslin, T. (2010). Overrun by the neighbors: landscape context affects strength and sign of local adaptation. *Ecology* *91*, 2253–2260.
48. Hanski, I. (2012). Eco-evolutionary dynamics in a changing world. *Ann. N Y Acad. Sci.* *1249*, 1–17.
49. Nosil, P. (2004). Reproductive isolation caused by visual predation on migrants between divergent environments. *Proc. Biol. Sci.* *271*, 1521–1528.
50. Sandoval, C.P. (1994). Differential visual predation on morphs of *Timema cristinae* (Phasmatodeae: Timemidae) and its consequences for host range. *Biol. J. Linn. Soc. Lond.* *52*, 341–356.
51. R Development Core Team (2012). R: A Language and Environment for Statistical Computing (Vienna, Austria) Available at: <http://www.R-project.org/>.
52. Pebesma, E. (2004). Multivariable geostatistics in S: The gstat package. *Comput. Geosci.* *30*, 683–691.
53. Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., and R Development Core Team (2012). nlme: Linear and nonlinear mixed effects models.
54. Sandoval, C.P. (2000). Persistence of a walking-stick population (Phasmatoptera: Timematodea) after a wildfire. *Southwest. Nat.* *45*, 123–127.
55. Rimmel, T., Davison, J., and Tammaru, T. (2011). Quantifying predation on folivorous insect larvae: The perspective of life-history evolution. *Biol. J. Linn. Soc. Lond.* *104*, 1–18.
56. Rimmel, T., and Tammaru, T. (2009). Size-dependent predation risk in tree-feeding insects with different colouration strategies: a field experiment. *J. Anim. Ecol.* *78*, 973–980.