

Observational evidence that maladaptive gene flow reduces patch occupancy in a wild insect metapopulation

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Theory predicts that dispersal throughout metapopulations has a variety of consequences for the abundance and distribution of species. Immigration is predicted to increase abundance and habitat patch occupancy, but gene flow can have both positive and negative demographic consequences. Here, we address the eco-evolutionary effects of dispersal in a wild metapopulation of the stick insect *Timema cristinae*, which exhibits variable degrees of local adaptation throughout a heterogeneous habitat patch network of two host-plant species. To disentangle the ecological and evolutionary contributions of dispersal to habitat patch occupancy and abundance, we contrasted the effects of connectivity to populations inhabiting conspecific host plants and those inhabiting the alternate host plant. Both types of connectivity should increase patch occupancy and abundance through increased immigration and sharing of beneficial alleles through gene flow. However, connectivity to populations inhabiting the alternate host-plant species may uniquely cause maladaptive gene flow that counters the positive demographic effects of immigration. Supporting these predictions, we find the relationship between patch occupancy and alternate-host connectivity to be significantly smaller in slope than the relationship between patch occupancy and conspecific-host connectivity. Our findings illustrate the ecological and evolutionary roles of dispersal in driving the distribution and abundance of species.

KEY WORDS: Connectivity, eco-evolutionary dynamics, extinction, gene flow, maladaptation, metapopulation.

There is now abundant evidence that rapid evolution can play a major role in driving ecological dynamics (Hairston et al. 2005; Schoener 2011), and many subdisciplines of ecology have seen excellent examples of the unexpectedly far-reaching effects of contemporary evolution (Bailey et al. 2009). For example, studies of population demography (Pelletier et al. 2007; Ozgul et al. 2010; Turcotte et al. 2013), predator-prey cycling (Yoshida et al. 2003; Becks et al. 2010; Hiltunen et al. 2014), community structure (Johnson et al. 2009; Farkas et al. 2013; Urban 2013; Pantel et al. 2015), and ecosystem function (Harmon et al. 2009; Bassar et al. 2010; Crutsinger et al. 2014) each support how

evolution has unanticipated explanatory power in an ecological context.

Eco-evolutionary research has for the most part focused on natural selection as the primary evolutionary process of interest, paying less attention to the effects of gene flow, mutation, and genetic drift. This focus has stunted the integration of rapid evolution into ecological subdisciplines for which dispersal is a key feature, such as metapopulation ecology (Hanski 2012), metacommunity ecology (Urban et al. 2008), and island biogeography (Farkas et al. 2015). Although natural selection should feature strongly in how evolution can be important for these subdisciplines (e.g., Farkas



et al. 2013; Urban 2013), a complete eco-evolutionary approach will necessarily integrate the role of gene flow that results from dispersal.

Metapopulation ecology in particular has been hugely successful as a paradigm with which to understand the dynamics of populations across space (Hanski 1998), forming a foundation for modern conservation biology (Hanski and Simberloff 1997). Studies integrating evolution into metapopulation ecology have largely focused on either the evolution of dispersal itself (Van Valen 1971; McPeck and Holt 1992; Ronce 2007; Amarasekare 2016), or on how natural selection and gene flow interact to drive the evolution of functional traits in heterogeneous environments (Hendry et al. 2002; Wynne et al. 2003; Kawecki 2004; Hanski et al. 2010; Hanski and Mononen 2011; Farkas et al. 2013). However, gene flow can either promote or constrain adaptation, depending on degrees of dispersal, habitat heterogeneity, local adaptation, and reproductive isolation (Garant et al. 2007). Therefore, the potential effects of dispersal on metapopulation dynamics as mediated by gene flow are complex, either increasing or decreasing the likelihoods of patch colonization and extinction. This contrasts with much metapopulation theory, which generally predicts dispersal, and population connectivity in particular, to increase patch occupancy through increased colonization of empty habitat and reduced extinction of occupied habitat (Hanski 1994; del Mar Delgado et al. 2011; but see Bowler and Benton 2005 for complexities). Hence, the eco-evolutionary effects of dispersal in metapopulations are complex, but little empirical work has tested a synthetic eco-evolutionary framework (Farkas et al. 2013).

One central but largely untested aspect of eco-evolutionary metapopulation biology is the prediction that gene flow can reduce patch occupancy through increased maladaptation. It is well documented that gene flow can cause maladaptation in nature (Riechert 1993; Storfer and Sih 1998; Moore and Hendry 2005; Postma and van Noordwijk 2005; Bolnick and Nosil 2007; Bolnick et al. 2008; McBride and Singer 2010; Paul et al. 2011; Fedorka et al. 2012; Kovach et al. 2015; Censer 2016), but the link from maladaptation to patch occupancy has not been well established empirically. In fact, evidence that maladaptive gene flow can have negative population demographic consequences at all is scarce, with support coming from only a small number of studies (Moore and Hendry 2009; Farkas et al. 2013).

In this article, we offer observational evidence that gene flow between divergently adapted *Timema cristinae* stick insect populations decreases the likelihood of local patch occupancy. In *T. cristinae*, strong selection by avian predators on a highly heritable camouflage polymorphism (striped vs. unstriped morphs) drives local adaptation to two morphologically dissimilar host-plant species (Sandoval 1994a; Nosil and Crespi 2006; Comeault et al. 2014; Gompert et al. 2014). However, gene flow between populations inhabiting alternate hosts breaks down local adap-

tation (Sandoval 1994b; Bolnick and Nosil 2007; Nosil 2009), leading to a geographic mosaic of (mal)adaptation (Sandoval 1994b; Bolnick and Nosil 2007). Variation in the local frequency of the poorly camouflaged morph ranges from as low as 1–3% and as high as 80%, due to variation in gene flow from populations adapted to the alternate host (Sandoval 1994b; Bolnick and Nosil 2007).

Recently, Farkas et al. (2013) reported results concerning two central predictions of eco-evolutionary metapopulation theory. First, they showed experimentally that camouflage maladaptation reduces *T. cristinae* abundance due to increased bird predation. Natural observations offered corroboration, showing that maladaptation negatively correlated with abundance. Second, they tested for an influence of gene flow on maladaptation by correlating the morph frequency in host-plant patches with the expected morph frequency of immigrants. They did not find an association, suggesting that gene flow did not play a role in structuring phenotypic variation across the landscape (Farkas et al. 2013). This finding was surprising given strong and diverse evidence for detectable effects of gene flow across multiple spatial scales in past work (Sandoval 1994b; Nosil et al. 2003, 2012; Nosil 2009). A third prediction—that maladaptive gene flow can reduce patch occupancy—remains untested empirically, although Farkas et al. (2013) used an eco-evolutionary metapopulation model (Hanski et al. 2010) to demonstrate how maladaptation in *T. cristinae* should in principle reduce habitat patch occupancy through increased local extinctions.

Here, we use the observational data from Farkas et al. (2013) to test this third prediction. To do so, we use the concept of population connectivity, which integrates the geographical distance to populations and abundance in those populations (see Supporting Information) to measure the expected amount of immigration to any given habitat patch (Hanski 1994). For every habitat patch, whether occupied or unoccupied, we can calculate population connectivity, and can calculate more derived and specific measures of population connectivity by excluding a subset of populations based on arbitrary criteria such as patch size or habitat type. Here, for each host-plant patch in the natural landscape of interest, we subdivide the total population connectivity by calculating two, nonoverlapping measures of connectivity based on host-plant species. “Alternate-host connectivity” measures population connectivity only to populations of *T. cristinae* living on the alternate host-plant species. “Conspecific-host connectivity” measures connectivity only to populations of *T. cristinae* living on the same (conspecific) host-plant species. For example, for all *Adenostoma fasciculatum* patches, conspecific-host connectivity measures population connectivity using only *T. cristinae* populations inhabiting other *A. fasciculatum* patches, whereas alternate-host connectivity measures populations connectivity only using *T. cristinae* populations inhabiting *C. spinosus* patches.

Metapopulation theory predicts increasing connectivity to increase patch occupancy due to ecological mass effects (i.e., direct increases in abundance through immigration), by increasing colonization and reducing the likelihood of extinction (Hanski 1998). However, evolutionary models predict more complex effects of connectivity for populations, especially in heterogeneous landscapes (Garant et al. 2007). On the one hand, gene flow can increase fitness as it promotes adaptation by increasing genetic variance (Barton 2001; Swindell and Bouzat 2006), facilitates the spread of beneficial mutations (Peck et al. 1998; Feder et al. 2003; Morjan and Rieseberg 2004), reduces genetic drift by increasing neighborhood size (Alleaume-Benharira et al. 2006), and reduces inbreeding depression (Ingvarsson and Whitlock 2000; Keller and Waller 2002). On the other hand, gene flow between alternate habitats may reduce mean population fitness and constrain adaptation by driving the phenotypic mean away from the optimum (Sandoval 1994b; Bolnick and Nosil 2007; Nosil 2009) and introducing a variance load as the distribution of phenotypes spreads away from the mean (Bridle et al. 2010). The positive fitness and demographic effects of gene flow and mass effects should be roughly equal for alternate- and conspecific-host connectivity, but we predict the negative effects to be larger for alternate- than conspecific-host connectivity (Fig. 1, inset).

Following the above-predicted mechanisms, we evaluate the influence of maladaptive gene flow by testing for a difference between the effects of alternate- and conspecific-host connectivity on patch occupancy and abundance (see Sexton et al. 2011 for similar approach). Because of the potentially negative effects of maladaptive gene flow on patch occupancy, eco-evolutionary metapopulation theory predicts the relationship between patch occupancy/abundance and alternate-host connectivity to be smaller in slope (i.e., less positive) than the relationship with conspecific-host connectivity (Fig. 1). The magnitude of difference in the slopes will be determined a balance of positive and negative effects. Although we might predict both slopes to be positive in sign, the sign of the slopes could differ if the effects of maladaptive gene flow are so strong that alternate-host connectivity has a net negative influence on patch occupancy, despite the positive role of mass effects and beneficial gene flow. In this context, conspecific-host connectivity serves as control against which we compare the effect of alternate-host connectivity, allowing us to isolate the effects of maladaptive gene flow on patch occupancy and abundance.

Methods

STUDY SYSTEM

Timema cristinae is a sexual, univoltine, flightless stick insect (Phasmida: Timematodea) endemic to a ~30-km² region of chaparral habitats outside Santa Barbara, California, where it is the

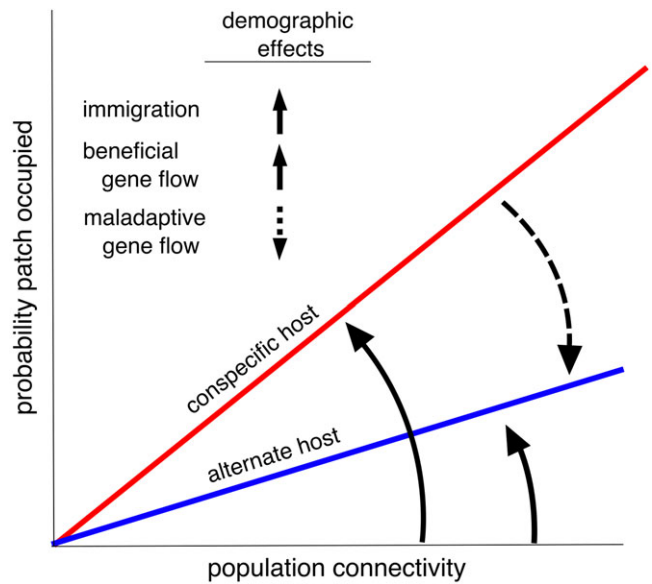


Figure 1. Predictions for effects of conspecific- and alternate-host connectivity on occupancy. The relationship between alternate-host connectivity and patch occupancy (blue, lower line) is expected to be smaller in slope than the relationship between conspecific-host connectivity and patch occupancy (red, upper line). Inset table shows demographic effects of processes associated with connectivity in heterogeneous landscapes. The solid arrows indicate positive demographic effects of immigration and beneficial gene flow, whereas the dashed arrow demonstrates negative demographic consequences of maladaptive gene flow. Relationships are drawn as linear for convenience, and are not meant to imply theoretically linear relationships.

only resident species in the genus *Timema*. They feed predominantly on the perennial shrubs *A. fasciculatum* (Rosaceae) and *Ceanothus spinosus* (Rhamnaceae), which are dominant plants in the chaparral of this region. The plant species differ substantially in leaf morphology, with *A. fasciculatum* having fascicled, needle-like leaves and *C. spinosus* having broad, ovate leaves. Divergent natural selection from bird predators has led to the evolution of two morphs that are each best camouflaged on one of the host-plant species: a striped morph with a prominent white dorsal stripe is best camouflaged on *A. fasciculatum*, and an unstriped, solid-green morph is best camouflaged on *C. spinosus* (Sandoval 1994a; Nosil and Crespi 2006). In addition, body shape, size, and hue are all under divergent selection from predators between the two host-plant species, and thus contribute to the ability of ecotypes to achieve good crypsis (Nosil and Crespi 2004, 2006). Mark-recapture studies demonstrate that many or even most *T. cristinae* individuals remain on a single host plant individual for extended periods, with the average weekly dispersal distance being ~2 m, the maximum ~8 m, and the per-generation dispersal estimated at ~12 m (Sandoval 2000). When the host-plant species are in proximity to one another, this dispersal can lead to

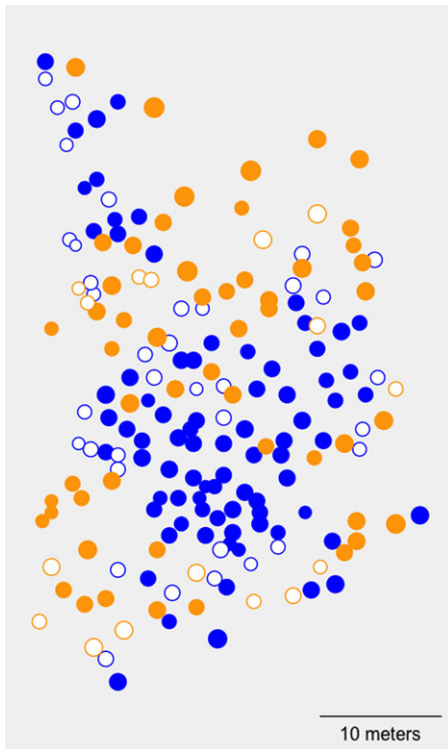


Figure 2. Map of host-plant patches inhabited by *T. cristinae* metapopulation. Blue (dark gray): *A. fasciculatum*, orange (light gray): *C. spinosus*, filled: occupied, unfilled: unoccupied. Circle sizes are scaled to \ln -transformed patch volume, calculated as a rectangular solid.

substantial degrees of maladaptation, depending on the relative abundance of each host species in the landscape (Sandoval 1994b; Bolnick and Nosil 2007).

SAMPLING PROTOCOL

Between the 1st and 5th of April 2011, we sampled all *A. fasciculatum* ($N = 117$) and *Ceanothus spinosus* ($N = 69$) patches in a 30×60 meter area (Fig. 2; N34.513297, W-119.800065, elevation: 850 m) by vigorously shaking all branches and catching fallen *T. cristinae* in a sweep net. Individual plants of the same species were determined to be part of the same patch if they were within 30 cm of one another. One of us (P.N.) phenotyped all *Timema* as striped or unstriped, recording morph frequencies and population sizes in each patch. Because melanic (brown, red, or gray) *Timema* individuals are relatively rare and are not host-associated (Comeault et al. 2015), records of melanic individuals were not included in calculations of abundance or phenotype frequency. We manually mapped the geographic location and measured the physical dimensions of each host-plant patch (Fig. 2), and patch size was calculated as rectangular solid. Further details of the sampling protocol are found in Farkas et al. (2013).

DATA ANALYSIS

The core test of our hypothesis involves testing for a difference between the effects of alternate- and conspecific-host connectivity on patch occupancy (occupied vs. unoccupied) and *Timema* abundance. We did this by combining generalized linear models with linear hypothesis testing (Fox and Weisberg 2011), comparing parameter estimates within the models. This approach is akin to testing for an interaction between the degree of connectivity and the type of connectivity (alternate vs. conspecific). However, each habitat patch has a measure of each type of connectivity, precluding the use of a two-way analytical approach. Alternate- and conspecific-host connectivity were calculated as connectivity to populations inhabiting the alternate versus conspecific host-plant species, the sum of which equals the total population connectivity (see Supporting Information for details of how these indices were estimated). Thus, alternate-host connectivity equals the connectivity to *T. cristinae* populations inhabiting *C. spinosus* for *A. fasciculatum* patches and *A. fasciculatum* for *C. spinosus* patches, whereas conspecific-host connectivity equals connectivity to *T. cristinae* populations inhabiting *A. fasciculatum* for *A. fasciculatum* patches and *C. spinosus* for *C. spinosus* patches.

For the analysis of both patch occupancy and abundance, we used a single hurdle model for zero-inflated and overdispersed count data (R package *pscl*; Zeileis et al. 2008) because it is a holistic approach that simultaneously analyses occupancy and abundance data, and because our abundance data were both overdispersed and zero-inflated, such that purely Poisson-error models were inappropriate (Supporting Information). The censored component of the model (zero vs. nonzero) used a binomial distribution with logit link function (analogous to a binomial GLM) and effectively modelled patch occupancy, whereas the zero-truncated component used a Poisson distribution and effectively modelled *T. cristinae* abundance, not including zero values.

In addition to considering the effects of connectivity on patch occupancy and abundance, we test for an influence of alternate- and conspecific-host connectivity on the degree of maladaptation, predicting a positive relationship with alternate-host connectivity, a negative relationship with conspecific-host connectivity, and a difference between the slopes of those relationships. We fit a generalized linear-mixed model using Penalized Quasi-Likelihood with Bernoulli error (MASS package in R), coding *Timema* as either adapted or maladapted, according to the match between their morph (striped vs. unstriped) and host-plant species, again employing linear hypothesis testing (Fox and Weisberg 2011) to compare slopes. Host-plant patch ID was included as a random factor ($n = 136$) to account for nonindependence among individuals collected from the same bush, but this factor explained negligible variance (intraclass correlation coefficient = 2.7×10^{-8}).

In both models (and both components of the hurdle model), we included alternate-host connectivity, conspecific-host connectivity, host-plant species, and ln-transformed host-plant patch volume (“patch size”) as independent variables. Following initial model fitting, we tested whether the parameter estimates for alternate-host connectivity and conspecific-host connectivity (1) differed from one another, and (2) as a sum were significantly different than zero, using Wald Chi-square comparisons in both cases (Fox and Weisberg 2011). The former tests the prediction that the effects of alternate-host connectivity on patch occupancy, abundance, and local adaptation is smaller than the effect of conspecific-host connectivity. The latter tests the predictions that total connectivity increases patch occupancy and abundance, but will have no effect on local maladaptation.

To check for a potential influence of spatial autocorrelation, which could violate the assumption of nonindependence and spuriously inflate power, we compared generalized linear models to penalized-quasilielihood generalized linear-mixed models incorporating spatial correlation structure for patch occupancy, *Timema* abundance, and maladaptation (Dormann et al. 2007). Results of PQL-GLMMs did not differ from those of GLMMs (see Supporting Information), indicating a lack of substantial spatial autocorrelation among residuals in our models. Accordingly, variograms showed no signature of autocorrelation for patch occupancy, and only slight autocorrelation at very small distances for abundance (see Supporting Information).

Results

PATCH OCCUPANCY AND *TIMEMA* ABUNDANCE

As predicted, the effects of alternate-host connectivity on both patch occupancy and *Timema* abundance were significantly smaller than those of conspecific-host connectivity (Fig. 3; occupancy: $\chi^2 = 11.91$, $P < 0.001$; abundance: $\chi^2 = 4.21$, $P = 0.040$). Patch occupancy significantly increased with conspecific host-connectivity, and showed a nonsignificant, negative trend with alternate-host connectivity (Table 1, Fig. 3A). *Timema* abundance increased with conspecific-connectivity and showed a nonsignificant positive trend with alternate-host connectivity (Table 1, Fig. 3B). It is important to highlight that the nonsignificance of alternate-host connectivity for patch occupancy and *Timema* abundance (Table 1) does not constitute evidence against a role for maladaptive gene flow. Effects of gene flow on occupancy and abundance simply predict a significantly lower slope for alternate- than conspecific-host connectivity, as we observed (Fig. 1, “Discussion”). Patch occupancy increased marginally with total population connectivity ($\chi^2 = 3.31$, $P = 0.070$), and *Timema* abundance increased significantly with total connectivity ($\chi^2 = 10.43$, $P = 0.001$). Both patch occupancy and *Timema* abundance increased with

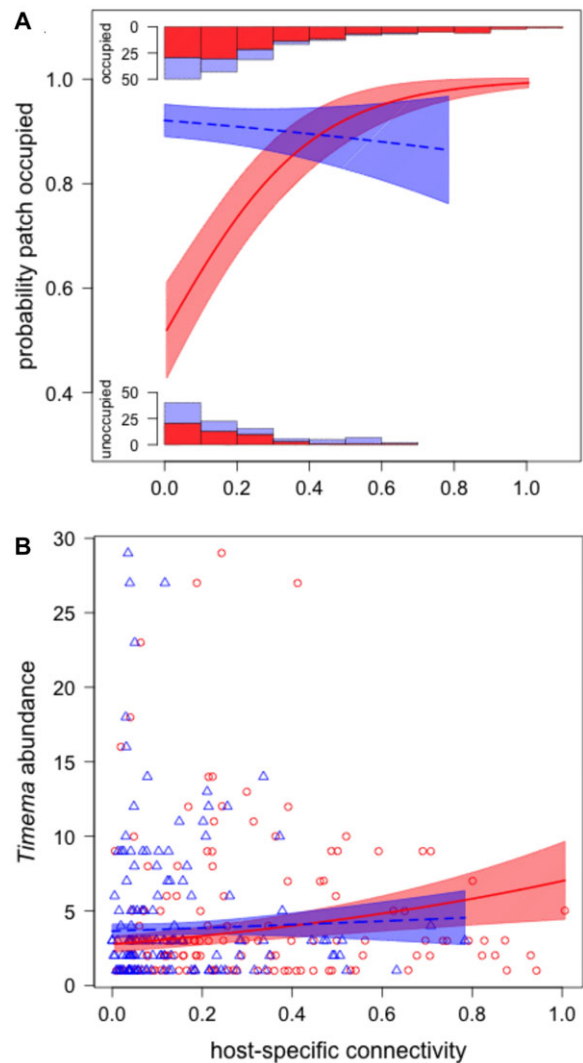


Figure 3. Relationships between connectivity and (A) patch occupancy and (B) *Timema* abundance. (A, B) Curves are predicted values for relationships between alternate-host connectivity (blue, dashed) and conspecific-host connectivity (red, solid), and shaded areas show ± 1 standard error. (A) Histograms show raw counts of occupied (top) and unoccupied (bottom) patches across levels of connectivity to alternate hosts (light blue) and conspecific hosts (dark red). (B) Points are raw, unconditional data for abundance and alternate-host connectivity (blue triangles) and conspecific-host connectivity (red circles). Predictions and errors are from generalized linear models of patch occupancy and *Timema* abundance (see Supporting Information).

patch size, but neither differed between host-plant species (Table 1).

MALADAPTATION

There were no significant effects of alternate- or conspecific-host connectivity on maladaptation (Table 1), no significant difference between effects of alternate- and conspecific-host connectivity on maladaptation ($\chi^2 = 0.22$, $P = 0.638$), and no significant effect of

Table 1. Effects of conspecific-host connectivity (Con-Host), alternate-host connectivity (Alt-Host), ln-transformed patch volume (Ln-Size), and host plant species (Host spp.) on patch occupancy (zero component in hurdle model), *Timema* abundance (count component of hurdle model), and maladaptation (Bernoulli GLMM).

Variable	Patch occupancy (zero model)				Abundance (count model)				Maladaptation			
	<i>b</i>	<i>se</i>	<i>z</i>	<i>P</i>	<i>b</i>	<i>se</i>	<i>z</i>	<i>P</i>	<i>b</i>	<i>se</i>	<i>z</i>	<i>P</i>
Con-Host	2.239	0.578	3.875	< 0.001	1.122	0.232	4.836	< 0.001	0.414	0.492	0.844	0.402
Alt-Host	-0.290	0.713	-0.406	0.685	0.475	0.344	1.382	0.167	0.071	0.730	0.097	0.923
Ln-Size	1.180	0.231	5.115	< 0.001	1.204	0.097	12.443	< 0.001	0.126	0.192	0.654	0.514
Host sp.	0.060	0.257	0.232	0.816	0.158	0.105	1.498	0.134	0.584	0.230	2.542	0.012

b, parameter estimate; *se*, standard error; *z*, *z*-value; *P*, *P*-value. Effects significant at $\alpha = 0.05$ are highlighted in boldface text.

total population connectivity ($\chi^2 = 0.23$, $P = 0.629$). Maladaptation was not influenced by patch size, but *C. spinosus* plants harbored significantly more maladaptation than *A. fasciculatum* plants (Table 1).

Discussion

For *T. cristinae* metapopulations, conspecific- versus alternate-host plant connectivities represent degrees of immigration from similar versus divergent habitat types. Coupled with local adaptation to those different habitats (Farkas et al. 2013), we predicted the demographic effects of alternate- and conspecific-host connectivity to differ due to a difference in the degree of maladaptive gene flow associated with immigration (Fig. 1). We show here that alternate-host connectivity has an effect on patch occupancy that is significantly smaller than that of conspecific-host connectivity ($P < 0.001$). This result helps fill a gap in eco-evolutionary metapopulation biology by providing evidence consistent with maladaptive gene flow reducing patch occupancy throughout a wild metapopulation.

As stressed above, significance of the negative correlation between alternate-host connectivity and patch is neither necessary nor sufficient to support the hypothesis. It is not sufficient evidence because the relationship between conspecific-host connectivity and occupancy could itself be negative, which would call into question the interpretation of a negative slope for alternate-host connectivity as supportive of maladaptive gene flow. It is not necessary evidence because immigration from *Timema* populations on the alternate host should have both a positive demographic effect of increased population size and beneficial gene flow, as well as a negative demographic effect of maladaptive gene flow (Fig. 1). It is possible that the balance between positive and negative effects weighs in favor of positive effects and leads to a significantly positive slope, but that would not in itself suggest that maladaptive gene flow plays no role in structuring patch occupancy. To test for a role of maladaptive

gene flow in driving patterns of patch occupancy, it is both necessary and sufficient to test for a difference between the slopes of these two measures of connectivity, where conspecific-host connectivity serves as a control against which to compare the effect of alternate-host connectivity (Fig. 1). A similar comparative analytical approach was used to demonstrate negative fitness consequences of maladaptive gene flow in *Mimulus laciniatus*, where experimentally induced gene flow from the species range core into the range margin increased fitness less strongly than gene flow between marginal populations (Sexton et al. 2011).

Evidence that maladaptive gene flow can reduce abundance is another prediction of eco-evolutionary metapopulation biology that has received little empirical support. Farkas et al. (2013) added support by showing that populations of *T. cristinae* harbouring maladapted morphs have lower abundances than well-adapted populations (see also Moore and Hendry 2009 for an example with stickleback). Here we use a stronger set of explanatory variables to confirm this result, finding a smaller effect of alternate-host connectivity on abundance than conspecific-host connectivity, paralleling the results for patch occupancy (Table 1, Fig. 3).

A third prediction is that gene flow can yield maladaptation. However, we found no relationship between either alternate- or conspecific-host connectivity and maladaptation (Table 1), and no significant difference between their effects. One possibility is that in our study network, gene flow is effective at a distance large enough relative to the spatial grain of heterogeneity that morph frequencies are smoothed or homogenised across large parts of the network. This process would result in some evidence for local adaptation at the scale of the entire network (Farkas et al. 2013), but could make effects of gene flow undetectable through a correlation of morph frequency and host-specific connectivity based on individual plants (Sandoval 1994b). However, because the demographic changes shown to be associated with host-specific connectivity in this study are in theory mediated by morph frequency, this explanation demands another mechanism for the link

between connectivity and demography. Instead, we suggest that gene flow does indeed cause maladaptation, but that differences in the temporal scale over which morph frequency, demography, and connectivity change leads to incongruent correlations. Maladaptation within *T. cristinae* populations can change rapidly throughout a season, on the order of days and weeks (Nosil et al. 2003; Nosil and Crespi 2006; Farkas et al. 2013; Gompert et al. 2014), whereas population connectivity values will be driven by the spatial occurrence, species identity, and volume of host plants (related to *Timema* abundance), which are relatively stable through time. Hence, the signal of maladaptive gene flow in morph frequencies should become quickly eroded as natural selection removes maladapted individuals from the population. This hypothesis explains why we would nevertheless expect to see lower population size and occupancy on patches experiencing high maladaptive gene flow—hard natural selection by avian predators removes many maladapted individuals, leaving populations better adapted, but with lower abundance.

Another possibility stems from range margin literature that shows how gene flow between divergently adapted populations along an environmental cline can lead to population collapse in the absence of a cline in local (mal)adaptation (Bridle et al. 2010; Polechová and Barton 2015). These studies show that the sharing of alleles for quantitative traits can lead to ubiquitous adaptation by increasing adaptive potential through increased genetic variance (Barton 2001). In these models, the demographic load associated with increased variance can reduce population sizes such that drift can overcome selection and lead to local extinction. The stripe (presence/absence or size) in *T. cristinae* is largely, but not completely, controlled by a few loci of large effect (Comeault et al. 2014, 2015), so this body of theory could perhaps help to explain our findings. However, the largely major-locus control of the stripe likely limits the usefulness of these models, which assume selection is distributed evenly across multiple loci, such that per locus selection is weak. With a trait driven by a few loci of major effect, gene flow between divergently adapted populations is less able to share beneficial alleles, and drift is less able to overcome selection when population sizes are low. Empirical findings that gene flow between divergently adapted populations causes maladaptation further suggest the sharing of beneficial alleles plays little role in promoting good camouflage (Sandoval 1994b; Bolnick and Nosil 2007; Nosil 2009).

A final possibility is that alternate-host connectivity causes maladaptive gene flow in traits that we did not measure in this study, and that are important enough to show a clear influence on abundance and occupancy. For example, body size and shape are known to be important for crypsis in addition to the dorsal stripe (Nosil and Crespi 2006), were not measured in this study, and may have been influenced by population connectivity. However, this hypothesis still leaves many things to be explained. First,

the fact that morph frequency is not influenced by connectivity remains enigmatic, given extremely strong support for the effects of gene flow on morph frequency in nature (Sandoval 1994b; Bolnick and Nosil 2007; Nosil 2009). Second, previous analysis shows that maladaptation correlates with *T. cristinae* abundance in this metapopulation (Farkas et al. 2013), so a revised hypothesis suggests that gene flow is a driver of demographic patterns acting through multiple traits simultaneously. Future research is needed to evaluate the potential role of maladaptation in traits other than the dorsal stripe, and to explain the why connectivity fails to predict morph frequencies.

The lack of geographical signal on morph frequencies reported here and in Farkas et al. (2013) remains enigmatic, and begs the question of whether alternate-host connectivity leads to gene flow per se, or whether it simply leads to the movement and change of phenotypes across space, without any genetic mixing between morphs through sexual reproduction. Although we do not have direct data showing that connectivity in the metapopulation studied here leads to genetic mixing, the existence of maladaptive gene flow in the *T. cristinae* system is well established in previous work examining a range of spatial scales in other populations. For example, genomic studies show that differentiation between populations is consistently greater (and linkage disequilibrium within populations lower) for populations geographically isolated from the alternative host, relative to populations adjacent to the alternate host (Nosil et al. 2003, 2012; Nosil 2007). In fact, genetic differentiation between adjacent populations is very weak (mean genome wide $F_{ST} \sim 0.01$; Nosil et al. 2012; Soria-Carrasco et al. 2014). Genomic data aside, classical genetic crosses within populations regularly segregate for both striped and unstriped morphs, consistent with a long history of gene flow at this locus between populations (Sandoval 1993; Comeault 2014; Comeault et al. 2015). Finally, levels of maladaptation are so high (e.g., over 80% of the maladapted morph) in some populations that it seems unlikely recurrent dispersal (without gene flow) could create them, year after year (Bolnick and Nosil 2007). A recent study in this system furthermore shows that the third, melanic *T. cristinae* morph might facilitate gene flow between populations inhabiting different host-plant species by preventing the expression of striped/unstriped phenotypes and thereby reducing selection pressure against maladapted genotypes (Comeault et al. 2015). Hence, substantial levels of gene flow in the metapopulation studied here are very likely to be contributing to the reported findings.

Our data provide insight into why studies might not always show a strong relationship between total population connectivity and patch occupancy, despite the strong prediction of metapopulation theory (Hanski 1998). Specifically, separately evaluating the effects of conspecific- and alternate-host connectivity demonstrated the value of considering the evolutionary importance

of population connectivity in heterogeneous environments. We showed that alternate-host connectivity does not correlate with either patch occupancy or *Timema* abundance, whereas conspecific-connectivity does so strongly in both cases (Fig. 2, Table 1). Hence, evaluating the relative roles of connectivity on ecology and evolution can be important because they might have contrasting effects on the ecological properties of populations, and could have downstream effects that influence community structure or ecosystem processes (Farkas et al. 2015). In this study, the net effect of connectivity is to increase patch occupancy and population size (but note that the effect on patch occupancy is only marginally significant). However, if a greater proportion of total connectivity were due to populations on the alternate host-plant species, or the effect of that connectivity were larger, the scales could be tipped such that the beneficial effects of connectivity might be nullified or even inverted. Increasing total connectivity could, in theory, reduce patch occupancy and population size due to maladaptive effects of gene flow (Farkas et al. 2015).

In addition to supporting major predictions of eco-evolutionary metapopulation biology, our results have potential relevance to the study of species range margins. A classic eco-evolutionary hypothesis in this discipline suggests that asymmetrical gene flow into species range margins from range cores with greater abundance constrains adaptation to novel environmental conditions at the margins, leading to failed marginal establishment and increased marginal extinction (Haldane 1956; Mayr 1963; Kirkpatrick and Barton 1997; Kubisch et al. 2014). Evidence supporting a role for gene flow in constraining species ranges shows either that gene flow may be asymmetric and hence flow from core to margin (Moeller et al. 2011; Paul et al. 2011), or that gene flow homogenizes phenotypes across environmental clines (Bridle et al. 2009; Emery et al. 2011; Fedorka et al. 2012). Until now, direct empirical evidence that maladaptive gene flow can prevent establishment or increase local extinction has been largely lacking from the range margin literature. However, in *T. cristinae*, local adaptation to host-plants in terms of crypsis exists outside the context of core-margin dynamics. Instead, the asymmetrical gene flow required by population genetics models predicting range boundaries (Kirkpatrick and Barton 1997) is determined externally by the size and spatial configuration of host-plant patches, rather than higher abundance at the species range core. Nevertheless, our results inform the range margin debate by modeling patch occupancy as a function of population connectivity, a measure that is largely a function of abundance.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Comparison of quasilielihood GLM and penalized quasilielihood GLMM for patch occupancy (1) and *Timema* abundance (2).

Figure S1. Variogram showing spatial autocorrelation among errors from GLMs for patch occupancy (a) and *Timema* abundance (b). Solid lines are LOWESS smoothers.