

# NATURAL SELECTION IN POPULATIONS SUBJECT TO A MIGRATION LOAD

Daniel I. Bolnick<sup>1,2</sup> and Patrik Nosil<sup>3,4,5</sup>

<sup>1</sup>Section of Integrative Biology, University of Texas at Austin, Austin, Texas 78712

<sup>2</sup>E-mail: danbolnick@mail.utexas.edu

<sup>3</sup>Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada

<sup>5</sup>E-mail: pnosil@zoology.ubc.ca

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Migration tends to oppose the effects of divergent natural selection among populations. Numerous theoretical and empirical studies have demonstrated that this migration–selection balance constrains genetic divergence among populations. In contrast, relatively few studies have examined immigration's effects on fitness and natural selection within recipient populations. By constraining local adaptation, migration can lead to reduced fitness, known as a “migration load,” which in turn causes persistent natural selection. We develop a simple two-island model of migration–selection balance that, although very general, also reflects the natural history of *Timema cristinae* walking-stick insects that inhabit two host plant species that favor different cryptic color patterns. We derive theoretical predictions about how migration rates affect the level of maladaptation within populations (measured as the frequency of less-cryptic color-pattern morphs), which in turn determines the selection differential (the within-generation morph frequency change). Using data on color morph frequencies from 25 natural populations, we confirm previous results showing that maladaptation is higher in populations receiving more immigrants. We then present novel evidence that this increased maladaptation leads to larger selection differentials, consistent with our model. Our results provide comparative evidence that immigration elevates the variance in fitness, which in turn leads to larger selection differentials, consistent with Fisher's Theorem of Natural Selection. However, we also find evidence that recurrent adult migration between parapatric populations may tend to obscure the effects of selection.

**KEY WORDS:** Gene flow, immigration, local adaptation, maladaptation, natural selection, selection differential, *Timema*.

Many species are composed of multiple populations that are distributed across ecologically heterogeneous landscapes. Environmental variation leads to divergent selection favoring different phenotypic adaptations in different locations, thus driving evolutionary divergence among populations (Endler 1986; Schluter 2000). However, migration tends to oppose divergence by generating gene flow that increases similarity among populations (Slatkin 1985; Lenormand 2002; Kawecki and Ebert 2004; Bridle

and Vines 2007). The resulting tension between migration and selection has two main consequences. First, migration tends to limit or prevent genetic and phenotypic divergence among populations. This idea is supported by extensive theory (Haldane 1930; Bulmer 1971a,b, 1972; Felsenstein 1976; Slatkin 1985; Hendry et al. 2001; Lenormand 2002; Kawecki and Ebert 2004) and numerous empirical tests (e.g., Riechert 1993; Sandoval 1994a; King and Lawson 1995; Storfer and Sih 1998; Hendry et al. 2001; Hendry and Taylor 2004).

A second consequence of migration–selection balance is known as “migration load” (Garcia-Ramos and Kirkpatrick 1997). This is the loss in mean fitness of a population that results from

<sup>4</sup>Present address: Zoology Department and Centre for Biodiversity Research, University of British Columbia, 6270 University Boulevard, Vancouver, B.C., Canada V6T 1Z4.

immigration of locally maladapted alleles. Migration load is analogous to the “mutation load” that arises when mutation inputs new alleles that, on average, are expected to be less fit than existing alleles. Although both migration and mutation have the potential to import locally beneficial novel alleles that promote adaptation (Kawecki 2000), immigrants from other environments may frequently carry alleles that are less fit in the local habitat. Consequently, in addition to constraining divergence among populations, migration displaces recipient populations from their local adaptive peaks. For Mendelian traits, this entails a reduction in the frequency of locally favored alleles that otherwise would be at or near fixation, whereas quantitative traits may be displaced from the mean value favored by selection.

Because natural selection purges less-fit immigrant genotypes from a population, stronger migration load (or mutation load) should coincide with strong, persistent directional selection. Consistent with this expectation, a number of studies have documented ongoing selection against immigrant genotypes or phenotypes (Hendry et al. 2001; D. Bolnick et al., in press; see also Nosil et al. 2005 for a review). For instance, water snakes on rocky islands in Lake Erie exhibit a high frequency of banded color patterns that are cryptic in vegetated habitats of the mainland, but are more vulnerable to predators along islands’ rocky shores. As a result, island populations exhibit persistent natural selection against banded morphs that are nevertheless reintroduced in each generation by new immigrants (King 1993; King and Lawson 1995). As a result of recurrent immigration, this persistent natural selection may not lead to any long-term changes in trait frequencies or means across generations (“selection response”). The maladaptive effect of immigration is thus revealed by higher selection differentials (within-generation changes in trait frequencies or means), rather than by measuring selection response. Note that in this article, we define selection differential as the unstandardized difference in trait frequencies within a population before and after a bout of selection. We reserve the term “selection intensity” for when the selection differential is standardized by the trait variance (Lande and Arnold 1983; Endler 1986). Migration load should be revealed by a persistently higher selection differential, but not necessarily a higher intensity.

To date, support for migration load has generally been confined to studies of individual populations, in which selection operates against immigrants (King 1992; Sandoval 1994a; Hendry et al. 2002; Moore and Hendry 2005). A more powerful approach is to compare the selection differential across multiple populations with varying levels of migration load. For instance, one might show that (1) greater immigration is associated with more maladaptation (Sandoval 1994a; Hendry and Taylor 2004; Nosil and Crespi 2004), and that (2) greater maladaptation leads to a stronger selection differential within each generation. To our knowledge,

the latter step has not previously been carried out. This article describes some simple theoretical predictions regarding these two trends, coupled with a comparative test of the predictions using morph frequency data from 25 populations of *Timema cristinae* walking-stick insects adapted to one of two host plant species.

### EMPIRICAL STUDY SYSTEM

*Timema* walking-sticks are wingless insects inhabiting the chaparral of southwestern North America (Crespi and Sandoval 2000). Individuals feed and mate exclusively on the hosts upon which they rest. *Timema cristinae* uses two distinct host plant species (*Ceanothus spinosus*: Rhamnaceae and *Adenstoma fasciculatum*: Roseaceae). A “population” of walking-sticks is defined as all of the insects collected within a homogenous patch of a single host plant species (as in Nosil et al. 2002, 2003). No study site had more than one patch of a given host species. “Parapatric” insect populations have a neighboring population using the alternative host plant. “Allopatric” populations are separated from all other populations adapted to the alternative host by distances > 50 times the 12 m per generation dispersal distance (Sandoval 1993, 2000). The regions between allopatric populations are occupied by unsuitable hosts. Sequence data from mitochondrial DNA, coupled with morphometric data, confirm that migration is very low between allopatric populations relative to migration between adjacent parapatric populations (Nosil et al. 2003; Nosil and Crespi 2004). For example, mitochondrial DNA differentiation is consistently lower between adjacent pairs of populations than between geographically separated pairs of populations (Nosil et al. 2003). Thus, by comparing allopatric and parapatric populations, we are able to contrast low-migration and high-migration settings. Note, however, that low rates of gene flow do not necessarily indicate a lack of immigrants, due to selection against less-cryptic immigrants (Nosil 2004; Nosil et al. 2005).

Immigration rates also vary among parapatric populations. Coalescent-based estimates of gene flow ( $m$ ) between parapatric population pairs range from 0.001 to 0.232, with a mean migration rate of 0.043 (Nosil et al. 2003). This variation in migration rates among parapatric populations is largely explained by variation in relative population sizes (inferred from host plant patch sizes, which are known to be correlated with insect population size, Sandoval 1994a). All else being equal, the immigration rate into a population is higher when the adjoining population is relatively large (Nosil et al. 2003).

Past work has shown that host-associated morphological divergence in *T. cristinae* is determined by a balance between selection and migration (Sandoval 1994a,b; Nosil 2004; Nosil and Crespi 2004). For instance, the degree of morphological divergence between populations is negatively related to levels of migration between them (Nosil and Crespi 2004). We focus here on

another trait that differs between host plant populations: striped versus unstriped color pattern (Sandoval 1993). Both of these “color-pattern morphs” exist within populations on each host, but the unstriped morph is more common on *Ceanothus* (mean frequency = 81%) whereas the striped morph is more common on *Adenostoma* (mean frequency = 72%; Sandoval 1994b; Nosil et al. 2002; Nosil 2004). Population divergence has occurred via differential visual predation: the unstriped color pattern confers crypsis and high survival on *Ceanothus* but low survival on *Adenostoma*, and vice versa for the striped pattern (Sandoval 1994a,b; Nosil 2004; Nosil and Crespi 2006). The color morphs are genetically determined (Nosil et al. 2007). Inheritance appears to be essentially one-locus Mendelian, with the unstriped pattern effectively dominant over striped, although variance in F2s suggests some small-effect modifier loci and dominance in the F1s sometimes appears incomplete (Sandoval 1993; Nosil et al. 2006a).

Migration in *Timema*, which are wingless, is thought to occur predominantly during the early part of the life cycle. Females drop eggs from the host they are resting on, onto the earth below. Eggs overwinter in the soil, hatch in January or February, and nymphs crawl to a host plant in which they are generally thought to remain resident for the remainder of their life (until June; Sandoval 1993; Sandoval 2000). In particular, 50% of individuals remain on the same plant for their entire life, and the average per generation movement distance is <12 m (Sandoval 2000). This average includes individuals that did not move from their original plant, and may miss rare long-distance dispersal events. There is one generation per year, and insects mature at roughly equal rates on both hosts, so similar developmental stages occurred on the two hosts at any sample period.

Given this life history, the homogenizing effects of migration are expected to be most obvious early in a generation, after which natural selection by visual predators presumably eliminates many immigrants. The selection differential can thus be estimated by contrasting the frequency of the locally cryptic morph in winter (shortly after migration) to the frequency in spring (after selection). We take advantage of this contrast to compare levels of maladaptation and selection differentials within and across populations with varying geographic settings. However, it should be noted that adult migration has not been conclusively ruled out. Adult dispersal would be most likely among parapatric populations, and would counteract the effects of selection within a generation.

### Model

We begin by presenting a few predictions of migration–selection theory, using a simple model of migration–selection balance between a pair of populations with divergent selection on a two-allele Mendelian trait. This model effectively encapsulates many of the features of parapatric pairs of *Timema* populations.

### MALADAPTATION AND SELECTION WITHIN INDIVIDUAL POPULATIONS

We assume that cryptic color patterns are determined by a single diploid locus with two alleles. Within any given population, there can thus be three possible genotypes: an adapted homozygote, a heterozygote, and a maladapted homozygote. Assuming random mating, at the start of each generation these genotypes have frequencies  $f_{AA}^i = p^2$ ,  $f_{AM}^i = 2p(1 - p)$ , and  $f_{MM}^i = (1 - p)^2$ , respectively, where the superscript  $i$  designates the population,  $A$  represents the adaptive allele (striped on *Adenostoma*, or unstriped on *Ceanothus*),  $M$  is the maladaptive allele, and  $p$  is the frequency of the adaptive allele. Immigration occurs early in the life cycle, changing the genotype frequencies to

$$f_{AA}^i(m) = f_{AA}^i(1 - m_{ji}) + f_{AA}^j m_{ji}, \quad (1a)$$

$$f_{AM}^i(m) = f_{AM}^i(1 - m_{ji}) + f_{AM}^j m_{ji} \quad (1b)$$

$$f_{MM}^i(m) = f_{MM}^i(1 - m_{ji}) + f_{MM}^j m_{ji} \quad (1c)$$

where  $m_{ji}$  represents the fraction of individuals in population  $i$  that immigrated from source population  $j$ . After immigration, the population may no longer be in Hardy–Weinberg equilibrium, which is why we focus on genotype rather than allele frequencies.

The population is then subject to selection favoring the locally cryptic morph, with fitness  $w_A = 1$ , and  $w_M = 1 - s$  for the locally cryptic and vulnerable morphs, respectively. In *Timema*, the unstriped morph is approximately dominant (see Sandoval 1993; Nosil et al. 2006a), so the fitness of the heterozygote will depend on which host population is being examined. We thus assign heterozygotes a fitness  $w_{AM} = 1 - hs$ , where  $h = 0$  on *Ceanothus* (where the dominant unstriped morph is cryptic), and  $h = 1$  on *Adenostoma* (where the recessive striped morph is cryptic). We emphasize that although  $h$  is used to reflect the effects of dominance, we give it only the values 0 and 1 to allow the following derivations to apply to a focal population on either host.

Given these fitness values, the frequencies of the three genotypes after selection are

$$f_{AA}(s) = \frac{f_{AA}(m)}{1 - hsf_{AM}(m) - sf_{MM}(m)}, \quad (2a)$$

$$f_{AM}(s) = \frac{(1 - hs)f_{AM}(m)}{1 - hsf_{AM}(m) - sf_{MM}(m)}, \quad (2b)$$

$$f_{MM}(s) = \frac{(1 - s)f_{MM}(m)}{1 - hsf_{AM}(m) - sf_{MM}(m)}. \quad (2c)$$

We then calculate the new allele frequencies from these surviving genotypes, and calculate the genotype frequencies in the subsequent generation, assuming random mating. This cycle of migration then selection was iterated until genotype frequencies approached equilibrium.

In principle, migration–selection balance in a two-island model can be solved analytically to find the equilibrium allele frequencies, level of maladaptation, and selection differential. For instance, Wright (1931) showed that the equilibrium allele frequency under migration–selection balance in a related model is

$$\hat{p}_i = 1 - \frac{1}{s_i} [m_{ji} (1 - p_j)] \tag{3}$$

for the allele favored by local selection, or

$$\hat{q}_i = 1 - \hat{p}_i = \frac{m_{ji} q_j}{s_i} \tag{4}$$

for the locally maladaptive allele. Wright’s result is quite intuitive: the frequency of the maladapted gene  $\hat{q}_i$  in a population should increase with the immigration rate, and decrease with selection against it. However, to make this analytical solution tractable, Wright had to make several simplifying assumptions: weak selection, even weaker migration, and a continent-island migration setting. These assumptions are unsuitable for *Timema* populations, where past studies have documented both strong selection and, in some instances, high migration rates (Sandoval 1994a,b; Nosil et al. 2003; Nosil 2004; Nosil and Crespi 2006). Furthermore,

there is no a priori reason to expect that migration and selection have greatly different magnitudes. Consequently, to obtain theoretical predictions suited to the *Timema* system we need to use numerical solutions that allow us to dispense with Wright’s simplifications.

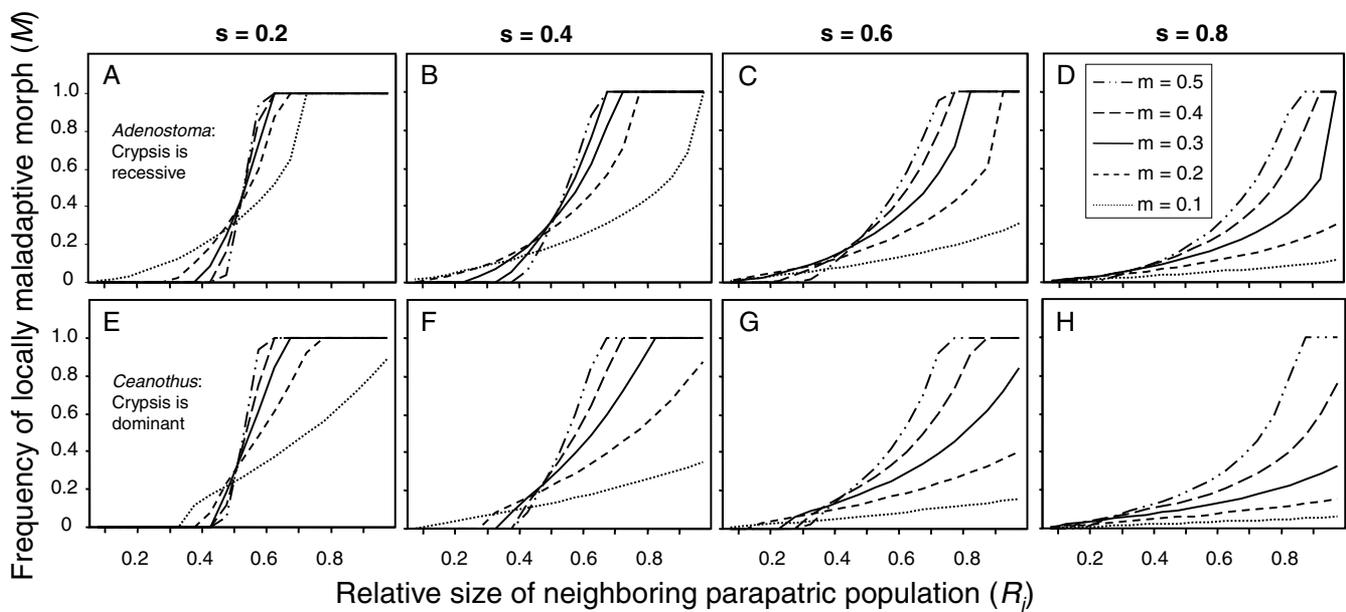
Deterministic iteration of migration (eq. 1) followed by selection (eq. 2) and random mating allowed us to determine how the equilibrium level of maladaptation depends on various parameters. In particular, we were interested in identifying the consequences of host plant identity ( $h = 0$  or  $1$ ), and of asymmetrical migration arising from unequal size of parapatric population pairs. A population with a relatively large neighbor is likely to have a higher immigration rate than a population with a relatively small neighbor. We therefore assume that

$$m_{ji} = cR_j, \tag{5}$$

where  $c$  is the average probability that an individual disperses, and  $R_j$  is the relative size of the neighboring population  $j$ ,

$$R_j = \frac{N_j}{N_i + N_j}. \tag{6}$$

When  $R_j \sim 0.5$ , the two populations are of roughly equal size and migration is symmetric. For  $R_j > 0.5$ , population  $i$  is relatively small and so receives a greater fraction of immigrants ( $m_{ji} > m_{ij}$ ). These asymmetries have a major impact on the equilibrium level of maladaptation within a population (Fig. 1). To better match our empirical results, we expressed maladaptation not in



**Figure 1.** The expected relationship between the degree of local maladaptation, and the relative size of the adjoining parapatric population ( $R_j$ , eq. 6), under an equilibrium two-island model of migration–selection balance. Maladaptation is measured as the frequency of the less-cryptic morph in a focal population. Separate panels are presented for four levels of selection ( $s$ ), and for the case in which the locally fit allele is recessive (*Adenostoma*, A–D), and dominant (*Ceanothus*, E–H). Within each panel, separate curves are given for varying levels of the mean migration rate, averaging  $m_{ji}$  and  $m_{ij}$ .

terms of allele frequencies, but as the frequency of the maladaptive morph,  $M = f_{MM} + hf_{AM}$ . Separate panels are provided for *Adenostoma* ( $h = 1$ ), and *Ceanothus* ( $h = 0$ ). Both cases conform to the intuitive expectation that populations with relatively large neighbors (large  $R_j$ ) tend to be more maladapted, as are populations with higher immigration or weaker selection. However, we note two other general trends. First, the degree of maladaptation is a concave function of population size asymmetry. Focusing on one population, maladaptation increases at a faster and faster rate as the neighboring population becomes relatively large. The accelerating level of maladaptation is particularly pronounced when selection is strong. Second, all else being the equal, the equilibrium level of maladaptation tends to be lower and the curve less concave in *Ceanothus*, where crypsis is dominant, than on *Adenostoma* (Fig. 1). In the empirical analyses described below, we therefore tested for curvature in the function relating maladaptation ( $M$ ) to relative population sizes ( $R_j$ ), and whether the function is lower and less curved on *Ceanothus*.

We next evaluated how the persistent maladaptation arising from immigration is related to the selection differential within populations. We represent the frequency of the locally adapted morph as  $A = f_{AA} + (1 - h)f_{AM}$ . Given the selection strengths outlined above ( $w_A = 1$ ;  $w_M = 1 - s$ ), the within-generation change in adaptive morph frequency due to selection is expected to be

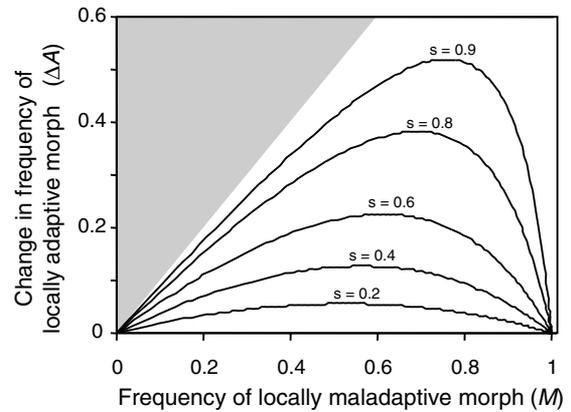
$$\Delta A = \frac{AMs}{1 - Ms} = \frac{s(M - M^2)}{1 - Ms}. \tag{7}$$

Note that our numerical iterations across generations used equation (2a-c) to track genotype frequencies, rather than using equation (7), because the latter equation is uninformative as to the genotypic composition of adaptive or maladaptive morphs. Note also that equation (7) holds only when  $h = 0$  or 1, as assumed here.

Assuming weak selection, equation (7) reduces to the familiar result that the selection differential is approximately proportional to the variance in fitness ( $\sim AM$ ), a relationship known as Fisher's Fundamental Theorem of Natural Selection (Fisher 1930). We expect to see little change in morph frequencies at very low or high frequencies of maladaptation, leading to a quadratic function (Fig. 2). A second insight is that at low levels of maladaptation (small  $M$ ), the slope of the curve in Figure 2 is approximately equal to the strength of selection,  $s$ . This can be seen by considering the derivative of equation (7)

$$\frac{d\Delta A}{dM} = \frac{s - 2Ms + M^2s^2}{(1 - Ms)^2}, \tag{8}$$

which approaches  $s$  as  $M$  gets small. Below, we use this relationship to empirically estimate the strength of selection ( $s$ ) against



**Figure 2.** The expected relationship between the within-generation selection differential and the degree of local maladaptation. The selection differential is measured as the within-generation change in frequency of the adaptive morph,  $\Delta A$ , and the degree of local maladaptation is the frequency of the less-cryptic morph. The shaded triangle represents disallowed regions of parameter space (e.g., a population with 90% adapted individuals cannot increase adaptation by more than 10%). Curves are presented for five strengths of selection against the less-cryptic morph ( $s$ ).

less-cryptic morphs in allopatric populations.

### MALADAPTATION AND SELECTION WITHIN PAIRS OF POPULATIONS

The trends documented in Figures 1 and 2 were obtained by focusing on maladaptation and selection differentials within a single member of a pair of populations. We next briefly present a novel result regarding the relative strength of selection in the two populations. Focusing on allele frequencies, at equilibrium the increased frequency of the adaptive allele due to selection must be exactly countered by the decreased frequency of the allele from immigration, so  $\Delta p(m)_i = m_{ji}(p_{t,i} - p_{t,j}) = -\Delta p(s)_i$  (Wright 1931). The ratio of selection differentials in the two populations is then

$$\frac{\Delta p(s)_i}{\Delta p(s)_j} = \frac{m_{ji}(p_{t,i} - p_{t,j})}{m_{ij}(p_{t,j} - p_{t,i})}, \tag{9}$$

which simplifies to

$$\frac{\Delta p(s)_i}{\Delta p(s)_j} = -\frac{m_{ji}}{m_{ij}}. \tag{10}$$

This equation shows that asymmetrical immigration rates lead to unequal selection differentials in a pair of populations. However, it also makes a more specific quantitative prediction that when one population experiences  $k$  times stronger immigration it will also exhibit a  $k$ -times stronger selection differential at equilibrium. If asymmetric migration rates are simply due to unequal population sizes (eqs. 5 and 6), the ratio of selection differentials further reduces to being the ratio of the population

sizes. The smaller population tends to be a net recipient of immigrants, and so is more maladapted and exhibits a larger selection differential.

## Materials and Methods

In the remainder of the article, we use a comparative approach to empirically evaluate the relationships between immigration, maladaptation, and selection described above. Over a five-year period from 2001 to 2005, P. Nosil measured the frequency of striped versus unstriped morphs in each of 25 populations of *T. cristinae* ( $n = 6661$  insects). Each population was sampled both early (January through March) and late (April to June) in the life cycle of a given generation. Insects were randomly sampled throughout the host plant patch using sweep nets. The specimens collected were used in other experiments and thus were not returned to the wild. Because the insects were removed randomly with respect to age, sex, and color, sampling is not expected to affect phenotype distributions in the population. Also, because sample sizes were small relative to total population sizes (perhaps about 1%), removal is not expected to affect population density or morph frequency. Some populations were sampled in multiple years, yielding a total of 59 estimates of changes in morph frequency between early and late periods of the life cycle (i.e., selection differentials,  $\Delta A$  from our model). Populations sampled in multiple years showed no decline in density. Sample sizes for each population and year are listed in online Supplementary Material, Appendix S1.

The degree of maladaptation in a given population was calculated as the frequency of the less-cryptic morph ( $M$ ) in the first sample of a given generation. The selection differential was calculated as the within-generation change in frequency of the more-cryptic morph for a population ( $\Delta A$ ): the frequency of the locally cryptic morph in the spring (late) sample minus its frequency in the winter (early) sample. This measure can thus have positive or negative values. Measurements of selection differentials by longitudinal comparisons between successive time periods in a generation tend to be conservative (Endler 1986). In particular, if immigration occurs simultaneously with selection, decreases in maladaptive morph frequency may be offset by new immigrants, leading to underestimates of selection. On the other hand, if maladapted individuals are more likely to emigrate as adults, our approach may overestimate the selection differential. Previous work has shown morph-specific host preferences are weak or nonexistent, suggesting that strong morph-specific emigration does not exist (Nosil et al. 2006a). We therefore stress that we are directly measuring “morph frequency change,” which is used to infer the selection differential. This inference is particularly reasonable in populations that experience little immigration (allopatric populations, or large parapatric populations that dwarf their neighbor), or if migration is restricted to an early life-history stage. We also

emphasize that we are not measuring the selection strength ( $s$ ), but the selection differential ( $\Delta A$ ).  $\Delta A$  is more suitable for the present study because theory predicts that immigration increases response to selection by elevating trait variance, rather than by increasing selection strength per se. However, estimates of  $s$  can be obtained by standardizing  $\Delta A$  by the morphological variance (Lande and Arnold 1983), or by the linear slope of a quadratic regression of  $\Delta A$  on  $M$  (eq. 7, Fig. 2).

Before proceeding with the main analyses (below), we confirmed that the striped morph is favored on *Adenostoma* and selected against on *Ceanothus*, as has been shown in previous studies using different sources and types of data (Sandoval 1994a,b; Nosil 2004; Nosil and Crespi 2006). A  $t$ -test evaluated whether the within-generation change in the frequency of the striped morph (percent striped in spring – percent striped in winter) differed between host plants. Positive values indicate selection for striped insects, negative values indicate selection against them (favoring unstriped insects). We also ran one-sample  $t$ -tests against a mean of zero separately for each host, to evaluate the null hypothesis of no morph frequency change. Both tests used exclusively allopatric populations in which recurrent migration throughout a generation is unlikely to confound our proxy for the selection differential. These  $t$ -tests were done using each yearly population survey as an independent observation. As a more conservative test, we repeated the  $t$ -tests using just the population as the level of replication (averaging across years for each population with multiple selection estimates). As explained below, we believe it is appropriate to treat selection responses as approximately independent observations because there were no significant population-specific effects on selection responses. In this and all analyses that follow, effects were treated as fixed effects.

For all the following statistical tests, assumptions of normality were evaluated with Kolmogorov–Smirnov one-sample tests for fit to a normal distribution (Lilliefors test), and homoscedasticity was evaluated by inspecting plots of residuals versus predicted values. Levels of maladaptation were not distinguishable from a normal distribution for allopatric populations (KS test statistic = 0.186,  $P = 0.067$ ), but selection differentials were moderately nonnormal in allopatry (KS = 0.213,  $P = 0.018$ ). In parapatry, maladaptation was moderately nonnormal (KS = 0.151,  $P = 0.024$ ), and selection differentials were roughly normal (KS = 0.117,  $P = 0.190$ ). Because ANOVAs and linear models are robust to moderate departures from normality (Lindman 1974) we did not carry out further data transformations (log transformation did not improve normality).

## MIGRATION AND MALADAPTATION

### I.—ALLOPATRY VERSUS PARAPATRY

Previous studies have found that maladaptation in *Timema* increases with the rate of immigration (Sandoval 1994a; Nosil and

Crespi 2004). Because parapatric populations experience more immigration than allopatric ones, we can double-check this result using an ANOVA with host plant species (*Ceanothus* vs. *Adenostoma*) and geography (allopatry vs. parapatric) as fixed effects and maladaptation (at the start of a generation) as a dependent variable. Not all of our estimates of maladaptation are statistically independent, because some populations were sampled in multiple years. Due to missing values (not all populations were sampled all years), we could not carry out a repeated measures ANOVA that would account simultaneously for variation among populations and among years. Instead, we used the average maladaptation (across years) for each population in our ANOVA, reducing our total number of replicates from 59 to 25. A second possible source of nonindependence can arise from genetic relatedness among populations. Genetic nonindependence is rarely considered in intraspecific comparative studies, particularly when populations are so closely related that gene trees exhibit paraphyletic/polyphyletic relationships, or when multiple genes yield inconsistent topologies suggesting there is reticulation (Rundle et al. 2000). Because our study populations exhibit such polyphyletic mitochondrial and nuclear relationships (Nosil et al. 2002, 2003), we treat them as independent units.

## MIGRATION AND MALADAPTATION

### II. – ASYMMETRIC IMMIGRATION IN PARAPATRY

Next, we tested whether maladaptation was greater in parapatric populations experiencing higher immigration rates (Fig. 1), taking advantage of varying levels of population size asymmetry. For any given focal population, we calculated the relative size of its neighboring patch ( $R_j$ , eq. 6), using the areas of the respective host plant patches. Patch sizes were measured using aerial photographs (Sandoval 1994a; Nosil et al. 2003). Previous work shows that host plant patch sizes are strongly correlated with both insect population size ( $r = 0.79$  and  $0.73$  for *Ceanothus* and *Adenostoma* patches respectively; Sandoval 1994a) and with maximum-likelihood estimates of the rate and the effective number of immigrants ( $m$ : Spearman's rank correlation  $r_s = 0.62$ , and  $Nm$ :  $r_s = 0.86$ ; Nosil et al. 2003). We then used a general linear model (GLM) to test whether maladaptation depends on the relative size of the adjacent population ( $R_j$ ), including host and host  $\times R_j$  interaction terms to evaluate whether the intercept or slope of this relationship differed between hosts. Note that this analysis excludes allopatric populations, and uses population mean maladaptation (across years) as the level of replication. In addition, we ran a quadratic regression to test our expectation that the relationship between maladaptation and relative population size is curvilinear, as predicted by theory (Fig. 1). Separate quadratic regressions also were run for each host type.

## MALADAPTATION AND SELECTION DIFFERENTIALS I.

### – ALLOPATRY

To test the expectation that maladaptation coincides with stronger selection differentials (Fig. 2), we regressed the initial level of maladaptation in a given year against the subsequent change in adaptive morph frequency. This regression used only allopatric populations, for which change in morph frequency should be a good proxy for the selection differential. The slope of the resulting function relating  $\Delta A$  to initial maladaptation  $M$  is used as an estimate of the strength of selection against less-cryptic morphs.

In this analysis, we treat repeated measures of each population as separate observations. In the previous analyses, we used population means across years because the explanatory variable (geography, relative population size) was invariant across years for a given population generating strong nonindependence. In contrast, the level of maladaptation varied substantially among years within a given population, making pseudoreplication less severe. To demonstrate this effective independence, we found no significant effect of population identity on levels of maladaptation in an ANCOVA ( $F_{5,21} = 1.054$ ,  $P = 0.413$ ), using populations for which we had three or more years of observations, and using the size of the adjoining population ( $R_j$ ) as a covariate. Note also that selection differentials observed in one year did not lead to lower maladaptation in successive years, further supporting our decision to treat different years' results as roughly independent instances of maladaptation's effect on selection differentials.

The high among-year variation in maladaptation and selection differential for a given population raises the question of whether the populations really are in a migration–selection equilibrium, as assumed in our model. Although we do not have sufficiently long-term surveys to conclusively demonstrate a quasi-equilibrium, we do note that the degree of maladaptation did not decrease significantly across years within any population. We posit that the among-year variation reflects a combination of sampling variance, and variation in the rate of migration or strength of selection across years. Consequently, our analysis assumes that maladaptation is fluctuating around a quasi-equilibrium value.

## MALADAPTATION AND SELECTION DIFFERENTIALS

### II. – PARAPATRY

Finally, we tested the relationship between morph frequency change and maladaptation in parapatric populations. If adult migration occurs between hosts, morph frequency change may tend to underestimate the effect of selection. This bias will tend to be most severe in populations adjacent to relatively large neighbors. Because these populations also tend to be more maladapted, recurrent immigration may obscure the relationship between selection differentials and maladaptation. We therefore used a linear model to test whether morph frequency change depends on maladaptation (a continuous variable), host plant, adjoining population size ( $R_j$ ),

and all interactions (pairwise and three-way). We used Akaike Information Criteria (AIC) coefficients (Akaike 1973) to select the linear model that best fits the data (using the GLM function in R), and then tested the significance of the terms in the optimal model using a GLM (in SYSTAT).

Because relative population sizes fell into four discrete clusters (Fig. 4), we categorized the neighboring population size as very small (1–6% of total parapatric pair), small (30–34%), large (61–70%), and very large (92–99%). Because trends were very similar between populations with very small and small neighbors, and between populations with large and very large neighbors, we further lumped relative population sizes into two categories: relatively small or relatively large neighbors and repeated the linear model, obtaining qualitatively similar results that are easier to visualize. These linear models were run using all 39 selection differential estimates for parapatric populations. As outlined above, this is likely justified given the high variation among years within populations. Note that these analyses also treat each population as an independent observation despite interactions between adjacent parapatric pairs. It would be preferable to analyze data based on population pairs, but few parapatric pairs were sampled within the same year. This prevents us from carrying out a direct test of equation (10), but also reduces the problem of nonindependence among paired populations.

## Results

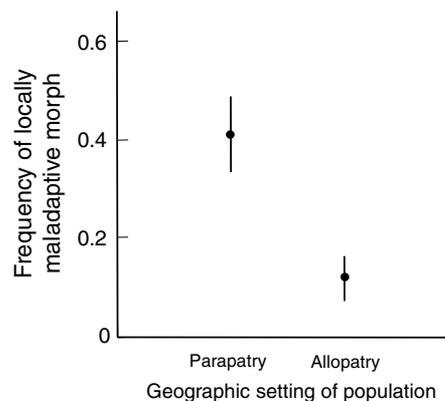
Consistent with results from previous studies, we found that striped morphs increased in frequency within a generation on *Adenostoma* (percent change =  $7.0 \pm 3.13$  SE) but decreased on *Ceanothus* (percent change =  $-3.3 \pm 1.55$  SE). These changes were significantly different from each other ( $t_{18} = 3.177$ ,  $P = 0.005$ ), although not quite significantly different from zero when considered separately ( $P = 0.089$  and  $0.054$ , respectively; but  $P < 0.05$  using Fisher's combined probability test). The comparison between hosts remained significant when using population averages as the level of replication ( $P = 0.01$ ).

### MIGRATION AND MALADAPTATION I. – ALLOPATRY VERSUS PARAPATRY

Parapatric populations exhibit a higher frequency of locally maladaptive (less cryptic) morphs than do allopatric populations (Fig. 3; ANOVA:  $F_{1,23} = 9.00$ ,  $P = 0.006$ ). This result confirms previous findings by Sandoval (1994a) and Nosil (2004).

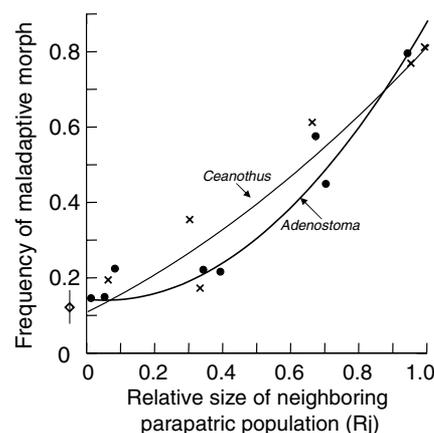
### MIGRATION AND MALADAPTATION II. – RELATIVE POPULATION SIZE IN PARAPATRY

Focusing on parapatric populations, we find that the degree of maladaptation increased with the relative size of the neighboring population ( $t = 9.873$ ,  $P < 0.001$ ,  $r^2 = 0.89$ ; Fig. 4, slope

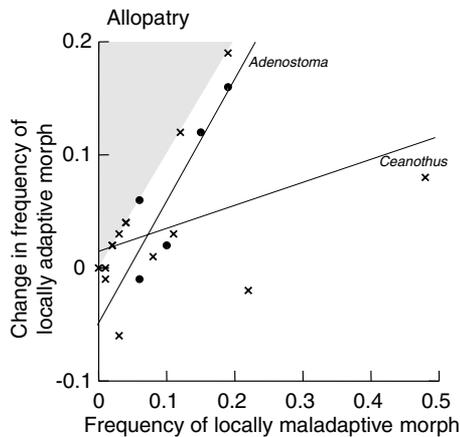


**Figure 3.** The degree of local maladaptation in parapatric versus allopatric populations ( $N = 15$  and  $10$ , respectively). Maladaptation is measured as the frequency of the locally less-fit morph (unstriped on *Adenostoma*, striped on *Ceanothus*). Error bars are  $\pm 1$  SE.

=  $0.686 \pm 0.069$  SE). This confirms a trend that has also been noted in previous studies (Sandoval 1994a; Nosil 2004; Nosil and Crespi 2004). However, we also find a new result: quadratic regression yields both significant linear ( $t = 5.462$ ,  $P < 0.001$ , slope =  $1.238 \pm 0.227$  SE) and quadratic terms ( $t = 2.518$ ,  $P = 0.029$ , slope =  $0.005 \pm 0.002$  SE). Although there is no significant host  $\times$  quadratic interaction ( $P = 0.58$ ), our power is low to detect differences in quadratic slopes between host populations. We do note, however, that there was a significant quadratic term in *Adenostoma* populations ( $P = 0.004$ ), but not in *Ceanothus* ( $P = 0.528$ ). Our results are thus consistent with the theoretical



**Figure 4.** The effect of population size asymmetry on maladaptation in parapatric populations of *Timema cristinae*. Populations with a relatively large neighbor ( $R_j > 0.5$ ) have a higher frequency of the locally less-cryptic morph. The mean ( $\pm 1$  SE) level of maladaptation in allopatric populations is shown to the left of the frame for reference. Symbols indicate whether the focal population inhabits *Adenostoma* (\*) or *Ceanothus* (X), and separate quadratic regression curves are shown for each host species.



**Figure 5.** The effect of maladaptation on populations' selection differentials in allopatry. Selection differentials are measured as the change in the frequency of the locally cryptic color morph from juveniles to adults within a generation. The shaded triangle represents disallowed regions of parameter space. Points that lie along the white/shaded interface represent the maximum possible response to selection. Symbols indicate whether the focal population inhabits *Adenostoma* (•) or *Ceanothus* (X). Separate linear regression lines are provided for each host.

prediction that there should be a concave function (Fig. 1), and hint at, but do not significantly support, our prediction of stronger quadratic slope on *Adenostoma*. On both hosts, the level of maladaptation appears to asymptote toward a level of maladaptation seen in allopatric populations, rather than toward zero.

## MALADAPTATION AND SELECTION DIFFERENTIALS

### I. - ALLOPATRY

The frequency of locally adapted morphs tended to increase between early and late samples within a year. In allopatry, the magnitude of this increase (i.e., the selection differential) is positively related to the initial frequency of the locally maladaptive morph (Fig. 5). This positive relationship is significant when using all selection estimates, treating different years from a given population as independent ( $t = 2.243$ ,  $P = 0.038$ ,  $r^2 = 0.218$ ). Using population means across years reduces our sample size to 10, resulting in a low power and nonsignificant trend, but in the same direction and with a similar slope and  $r$ -squared value ( $t = 1.78$ ,  $P = 0.112$ ,  $r^2 = 0.285$ ). However, under the reasonable assumption that crypsis is adaptive, a one-tailed test would apply and the regression with population means approaches significance ( $P = 0.056$ ).

There is weak evidence that selection differentials differ between host plants: a GLM with both host and maladaptation as effects found an interaction term that approached significance ( $F_{1,16} = 3.354$ ,  $P = 0.086$ ), although without main effect of host ( $F_{1,16} = 1.173$ ,  $P = 0.295$ ). The interaction term reflects a generally lower slope for *Ceanothus* (slope =  $0.206 \pm 0.12$  SE) than for

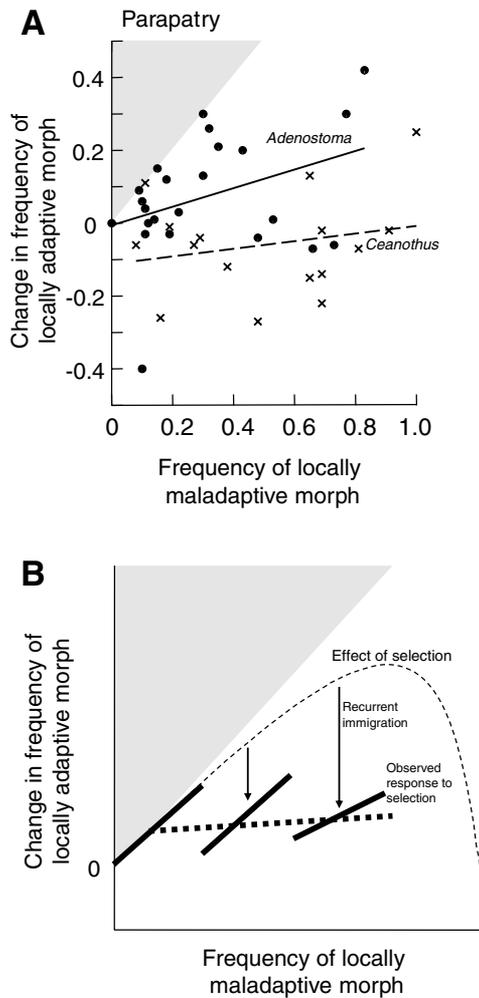
*Adenostoma* (slope =  $1.086 \pm 0.33$  SE). There was no significant quadratic term for either host type alone, but quadratic regression of the pooled data found both significant linear (slope =  $0.877 \pm 0.294$  SE,  $P = 0.008$ ) and quadratic terms (slope =  $0.014 \pm 0.006$  SE,  $P = 0.039$ ). The significant quadratic term is consistent with the prediction illustrated in Figure 2. However, it is driven entirely by a single highly maladaptive outlier in *Ceanothus* (Fig. 4, with the outlier removed the quadratic term has  $P = 0.381$ ) and so should not be overinterpreted.

As pointed out in the model section, the linear coefficient of the quadratic regression model yields an approximate estimate of the strength of selection ( $s$ ). We therefore estimate that there is remarkably strong selection against less-cryptic morphs in *Timema* ( $s \sim 0.877$ ). This strong selection is seen intuitively by noting that many of the changes in morph frequencies fall very close to the maximum  $\Delta A$  (Fig. 5).

### MALADAPTATION AND SELECTION 2. - PARAPATRY

At first glance, parapatric populations do not show a significant effect of maladaptation on the change in adaptive morph frequency ( $t = 0.807$ ,  $P = 0.425$ ,  $r^2 = 0.017$ , Fig. 6A), unlike the results from allopatric populations. The most likely explanation for the difference between allopatric and parapatric regressions is that in parapatry, recurrent immigration between sampling periods may obscure selection against immigrants that operates over the same time period (Fig. 6B). Such a recurrent immigration is likely to be more substantial in populations that adjoin much larger neighboring host patches. We therefore incorporated relative population size into our analysis.

A significant overall effect of maladaptation on subsequent morph frequency changes was recovered by including relative population size as a categorical variable in a linear model (Table 1), thereby yielding results consistent with those from allopatric populations. The positive relationship between maladaptation and selection differentials holds whether we lump the size of the adjoining population into two or four categories ( $P = 0.002$ ,  $0.048$ , respectively). AIC model selection criteria and GLM hypothesis tests confirm that host, relative patch size, and level of maladaptation contribute to variation in selection differentials in parapatric populations (Table 1). To make it easier to visualize these trends, we focus on the two-category approach, represented in Figure 7. This figure shows four separate regression lines relating morph frequency change to maladaptation, one line for each combination of host plant and relative population size (categorized into  $R_j >$  or  $< 0.5$ ). There is a significant positive relationship between morph frequency change and maladaptation in *Adenostoma* populations with a small neighbor ( $P = 0.012$ ) and in *Ceanothus* populations with a large neighbor ( $P = 0.038$ ). Note that in the latter case, the positive relationship exists even though the datapoints are far away from the theoretical boundary (shaded triangle, Fig. 7).



**Figure 6.** (A) The overall effect of maladaptation on populations' selection differentials in parapatry when relative population sizes are not accounted for (see Fig. 7). No significant trend exists (*Ceanothus*:  $t = 0.853$ ,  $P = 0.408$ ; *Adenostoma*:  $t = 1.832$ ,  $P = 0.081$ ), but there is an overall lower differential on *Ceanothus* (X) than on *Adenostoma* (•) ( $P = 0.001$ , Table 1). (B) A conceptual model to explain the apparent lack of relationship between selection differentials and maladaptation in parapatry. The actual rate at which selection removes maladaptive morphs (thin dotted line) increases with greater maladaptation, up to a point where low genetic variation limits differentials at high levels of maladaptation. However, recurrent gene flow within a generation counteracts morph frequency changes due to selection, so that the observed differentials (thick lines) are displaced downward. Because recurrent gene flow is more severe for relatively small populations, which are also more maladapted, the observed gene frequency changes are displaced downward to an increasing degree as one moves from left to right along the x-axis (arrows). Consequently, no trend would be detected if one were to ignore the effects of recurrent migration and run a single regression line through all datapoints (thick dotted line), even though a relationship between maladaptation and selection differential is maintained when one accounts for relative population sizes (thick solid lines).

**Table 1.** General Linear Model results regarding the effects of maladaptation, host, and relative size of the adjoining patch ('size'), on the change in the frequency of the locally cryptic morph within parapatric populations. Relative population size is categorized into either 2 categories or 4 categories (see text for explanation). Bold font indicates significant effects at  $P < 0.05$

# of population size categories	Factor	df	MS	F-ratio	P
2 categories					
	Maladaptation	1	2131.4	10.71	<b>0.002</b>
	Host	1	2703.1	13.59	<b>0.001</b>
	Size	1	1357.4	6.82	<b>0.013</b>
	Error	35	199.0		
4 categories					
	Size	1	496.2	2.60	0.075
	Host	3	655.3	3.44	0.073
	Maladaptation	1	821.4	4.31	<b>0.048</b>
	Host*Size	3	301.8	1.58	0.217
	Maladaptation*Size	3	487.4	2.56	0.076
	Error	27	190.7		

This highlights the fact that the positive relationship between maladaptation and selection differentials is not imposed simply by a limitation on the parameter space in which the data can fall.

*Adenostoma* populations with a large neighbor also show a positive relationship similar in slope to *Adenostoma* populations with a small neighbor, although the relationship is not significant in this case ( $P = 0.322$ ; Table 2). The one exception to the expected positive trend comes from *Ceanothus* populations adjoining a smaller neighbor, which show no significant relationship but tends toward a negative slope ( $P = 0.69$ , Table 2, Fig. 7B). Even more surprisingly, the *Ceanothus* populations with small neighbors exhibit a generally negative selection differential, with an increase in maladaptation within a generation. Because neither AIC nor a full-model GLM (not shown) supported the inclusion of a three-way interaction term, this one exception is not statistically different from the three other slopes. An additional linear model using just the parapatric *Ceanothus* populations found no significant difference in slope between populations with large versus small neighbors (Fig. 7B, size  $\times$  maladaptation interaction  $F_{1,12} = 3.18$ ,  $P = 0.10$ ). We therefore conclude that, in general, greater maladaptation leads to a stronger selection differential, after one accounts for the bias introduced by relative population size (Fig. 6B). To illustrate this bias, consider the contrast between large and small *Adenostoma* populations (Fig. 7A): their slopes are both positive and not significantly different from each other, but the intercepts are quite different.

In addition to the significant effect of maladaptation, we also observed significant effects of relative population size and host

**Table 2. AIC model selection criteria for the analysis of the effects of maladaptation, host, and relative size of the adjoining patch ('size'), on the change in the frequency of the locally cryptic morph within parapatric populations. Relative population size is categorized into either 2 categories or 4 categories (see text for explanation). The line in bold text indicates the preferred model**

AIC coefficients Model	2 size categories	4 size categories
Mal + Host + Size + Mal*Host + Mal*Size + Host*Size + Mal*Host*Size	325.7	327.2
Mal + Host + Size + Mal*Host + Mal*Size + Host*Size	327.2	329.1
Mal + Host + Size + Mal*Host + Mal*Size	325.4	329.4
Mal + Host + Size + Mal*Host + Host*Size	326.0	332.8
Mal + Host + Size + Mal*Size + Host*Size	325.4	<b>327.1</b>
Mal + Host + Size + Mal*Host	324.1	330.9
Mal + Host + Size + Mal*Size	323.5	327.4
Mal + Host + Size + Host*Size	324.9	324.9
Mal + Host + Size	<b>322.9</b>	332.2
Mal + Host +	331.3	327.8
Mal + Size	327.8	339.1
Host + Size	333.7	333.5
Mal	329.6	335.5
Host	336.2	329.6
Size	335.5	337.6

plant (Table 1B,D), although these effects were marginally significant when neighboring population size was classified into four categories. *Adenostoma* populations exhibited consistently stronger selection differentials than *Ceanothus* (Fig. 6). The relative population size effect occurred because, for a given level of maladaptation, populations with relatively small neighbors showed a larger selection differential than populations with large neighbors.

## Discussion

This study provides comparative support for the idea that immigration coincides with stronger selection differentials, consistent with previous evidence for selection against immigrants within individual populations (King 1993; King and Lawson 1995; Hendry et al. 2001; Nosil et al. 2005; D. Bolnick, in press). We have presented a comparative analysis using 59 estimates of selection differentials obtained over a five-year period from 25 populations of *Timema* walking-sticks. Our empirical results establish two major qualitative trends. First, populations receiving more immigrants tend to be more maladapted, confirming the results of previous studies (Sandoval 1994a; Nosil et al. 2003; Nosil 2004). The positive relationship between immigration and maladaptation occurs when contrasting allopatric and parapatric populations, and also when contrasting parapatric populations of varying size relative to their neighbor. Because migration rate is the main feature that distinguishes parapatric and allopatric populations, it is very likely that migration is the cause of the maladaptation.

Second, we present a novel finding that increased maladaptation coincides with a larger selection differential, as measured

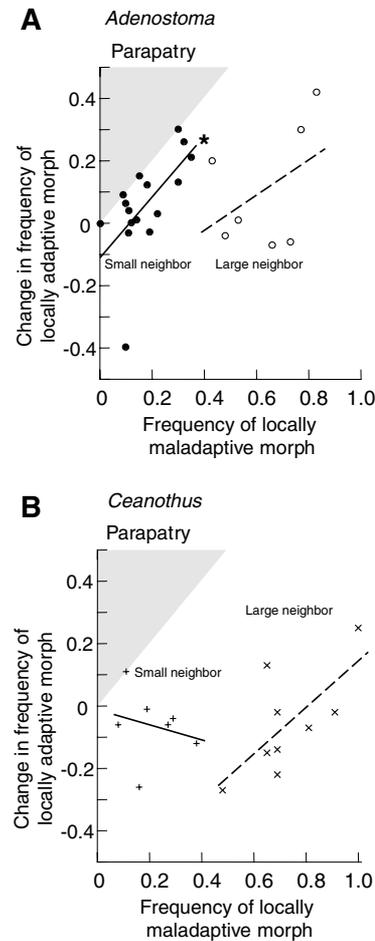
by the change in morph frequency within a given generation. At low frequencies of maladaptive morphs, increased adaptation is necessarily small (shaded area, Fig. 5). Consequently, as long as a trait is subject to selection, its rate of change necessarily increases with trait variance, consistent with Fisher's Fundamental Theorem of Natural Selection (Fisher 1930), and with our own model (eq. 7). Although Fisher's theorem is uncontroversial, quantitative demonstrations of this relationship are lacking for natural populations. Figures 5 and 7 thus represent a rare illustration of the theorem using comparative analysis of selection in nature. Although theory predicts a quadratic relationship between maladaptation and selection, we did not see strong empirical evidence for a negative quadratic function (Fig. 2 vs. Fig. 5). This is easily explained by the fact that in allopatric populations, our data were restricted to one end of this quadratic function (low levels of maladaptation), and hence approximate a linear relationship.

We also observed a positive relationship between maladaptation and selection differential in parapatric populations. The positive correlation was not initially obvious, but was significant once we accounted for the overall tendency of populations to exhibit weaker selection differentials due to relative population size, presumably reflecting recurrent migration throughout the generation (Fig. 6B). Such recurrent immigration would help explain why parapatric populations do not exhibit significantly larger selection differentials than allopatric populations.

We wish to emphasize that the positive relationship illustrated in Figure 5 is biologically informative, rather than being simply a trivial trend imposed by restrictions on the distribution of data (shaded area). First, it is quite possible to obtain a flat or negative

trend, for example if selection is absent, weak, or variable. Decreased adaptation was in fact observed in some populations in some years (Figs. 5, 7). Second, the fact that we observe the positive relationship between maladaptation and selection differentials in parapatry is important because most of the curves relating maladaptation to selection differentials are displaced away from the constraining mathematical boundary (shaded area, Fig. 7), due to higher overall levels of maladaptation. Hence the positive relationship is not imposed merely as a result of the boundary. Finally, the slope of the relationship between maladaptation and morph frequency change provides some novel biological insight, in the form of a rough estimate of the selection strength,  $s$ . It is surprising how often the rate of adaptation in *Timema* approached its mathematical upper limit, with ending allele frequencies each generation very close to 100% cryptic. This indicates that there is very strong natural selection for crypsis, which can be estimated by the linear term of a quadratic regression of  $\Delta A$  on  $M$  (Fig. 5,  $s \sim 0.877$ , pooling all allopatric populations). Previous experimental estimates of selection also found evidence of strong selection (e.g.,  $s = 0.53$  in Nosil 2004; see also Nosil and Crespi 2006). The slightly higher value of  $s$  in the present study may reflect differences in the time span over which selection occurred, or perhaps some agents of selection were less effective in the experimental than in the natural setting.

It is possible that some of the morph frequency change we documented is a result of forces other than natural selection, such as migration. In particular, if individuals are more likely to emigrate from a host in which they are maladapted, this recurrent adult migration could bias our estimates of selection. However, previous studies have found that morph-specific host plant choice in *T. cristinae* is absent or weak at best (Nosil et al. 2006b). Different morphs within allopatric populations do not exhibit any difference in host preference, indicating that a pleiotropic effect of color-pattern loci on host preference is unlikely. Furthermore, although emigration of less-cryptic individuals might



**Figure 7.** Effect of maladaptation on populations' selection differentials in parapatry when relative population sizes are accounted for. The shaded triangle represents disallowed regions of parameter space. The asterisk (\*) indicates a significant regression line ( $P < 0.05$ ). (A) *Adenostoma* populations, subdivided into those whose neighbor is relatively small ( $\bullet$ , solid line) and large ( $\circ$ , broken line). (B) *Ceanothus* populations, subdivided into those whose neighbor is relatively small ( $+$ , solid line), and large ( $\times$ , broken line). See Table 3 for statistical results.

**Table 3.** Regressions testing the effect of maladaptation on the change in morph frequency, within each combination of the adjoining population size and host categories. Shown are test statistics ( $t$ ), significance levels ( $P$ ), intercepts, and slopes, and corresponding standard errors (SE). The adjoining patch size is categorized into two levels: large and small ( $R_j > 0.5$  and  $< 0.5$ , respectively). Categorizing size into four levels leaves too few points within each host  $\times$  size combination to perform individual regressions, although the overall general linear model gives equivalent results. Bold font indicates slopes (or intercepts) significantly different from zero. Table 1 provides the statistical results for analyses pooling all four categories listed here.

Adjoining					
Host	Patch Size	$t$	$P$	Intercept (SE)	Slope (SE)
Adenostoma	Large	2.89	0.012	-11.08 (6.71)	<b>0.976</b> (0.34)
Adenostoma	Small	1.10	0.322	-24.88 (33.31)	0.565 (0.51)
Ceanothus	Large	-0.53	0.619	-1.19 (10.64)	-0.214 (0.46)
Ceanothus	Small	2.6	0.038	<b>-59.94</b> (21.65)	<b>0.743</b> (0.291)
Pooled into one regression		0.807	0.425	-1.18 (4.71)	0.079 (0.098)

have an effect in parapatry, it presumably would not occur in allopatric populations in which alternative hosts are not available. However, we find equally strong morph frequency changes in allopatry as in parapatry (ANOVA testing for a geography effect on  $\Delta A$ :  $F_{1,55} = 1.16$ ,  $P = 0.286$ ; ). We therefore conclude that morph-specific emigration is not contributing significantly to the observed selection differential.

Our data also support a novel and fairly specific prediction arising from our model of migration–selection balance. Theory predicted that the relationship between asymmetric migration and maladaptation should have a positive quadratic curvature, particularly pronounced in *Adenostoma* in which the locally adaptive striped morph is recessive. Our empirical data supported this quadratic relationship, indicating that levels of maladaptation will be more sensitive to changes in relative population size for smaller populations, than in larger populations. This match between our data and theory supports the overall utility of the simple two-island model in describing the dynamics of migration–selection balance in parapatric population pairs of *Timema*. Considering the two host types separately, we found a significant positive quadratic term in *Adenostoma* but not in *Ceanothus*, although the curvatures were not significantly different from each other. If this difference in curvature between hosts is indeed real, it would confirm that genetic dominance has a weak quantitative influence on the outcome of migration–selection balance. A second quantitative prediction from our model was that differences in selection differentials are proportional to differences in immigration rates (eq. 10). We were unable to test this prediction because we did not have allele frequency data, or enough concurrent selection estimates in adjacent pairs of parapatric populations.

Our empirical results highlight several other interesting aspects of *Timema* natural history that may warrant further study. First, the allopatric populations are not fixed for the locally adapted allele, yet exhibit strong selection that eliminates locally less-cryptic morphs. The persistence of maladaptive individuals could be explained by three hypotheses, which are not mutually exclusive. First, there may be regular immigration even into allopatric populations, supported by the fact that postselection phenotype frequencies were often close to 100%, yet the maladaptive morph was present again early in subsequent years. Although molecular analyses suggest that allopatric populations are genetically divergent from other conspecific populations, this evidence for weak gene flow does not rule out regular immigration if selection is strong (Nosil et al. 2005). Second, predation may be frequency dependent due to search image formation by predators, maintaining the less-cryptic morph at low frequencies (Bond and Kamil 2002). Third, epistasis may help shelter some genetic variation because stripe expression is conditional on the genotype at an independent locus controlling overall body color (Sandoval 1993; Nosil et al. 2006a). On *Adenostoma*, the loss of the lo-

cally maladapted allele could also be delayed by its recessive expression, although this explanation would not apply to *Ceanothus* populations.

Another pattern worth noting is that populations with relatively large neighbors tend to exhibit weaker selection differentials than we would expect given their maladaptation (Fig. 7). We therefore suggest that migration between parapatric host populations continues throughout a generation, rather than being restricted to nymphs. Such recurrent immigration also helps explain some of the negative selection differentials, in which locally adapted morphs declined in frequency between sampling periods. This finding highlights the importance of accounting for the relative timing of migration and selection, and the conservative nature of longitudinal selection estimates.

Finally, we find some evidence that morph frequency change in parapatry is generally smaller on *Ceanothus* than *Adenostoma* (Fig. 6), which can be explained in two ways. First, selection may be weaker on *Ceanothus*. This hypothesis is not strongly supported by our estimates of selection differentials in allopatric populations: the two hosts show similar overall differentials (ANOVA:  $F_{1,18} = 1.335$ ,  $P = 0.263$ ), but show marginally different slopes in Figure 5. It is also contrary to experimental results in which similar selection strength is observed on each host (Sandoval 1994a,b; Nosil 2004; Sandoval and Nosil 2005; Nosil and Crespi 2006). A second hypothesis is that there is a directional bias to migration between hosts, with greater flux from *Adenostoma* onto *Ceanothus*. Biased migration is supported by extensive evidence that *Adenostoma* populations are more willing to accept *Ceanothus* as a host than vice versa. In fact, individuals from both types of populations often prefer to rest on *Ceanothus* (Nosil et al. 2006a,b), although *Ceanothus* populations exhibit a stronger preference. Asymmetric preference strengths may reflect the fact that fecundity in the absence of predation is generally higher for insects raised on *Ceanothus* (Sandoval and Nosil 2005). We therefore suggest that the lower overall selection differentials in parapatric *Ceanothus* populations are a result of greater recurrent immigration due to a directional bias in movement arising from asymmetric host preference.

In conclusion, our comparative analyses of morph frequency changes in *Timema* walking-stick insects provide strong support for the idea that immigration leads to increased maladaptation, which in turn causes a persistent directional selection. We also highlight the value of asymmetric migration in studying the impact of migration load on populations. Such migration load has a number of important consequences. For instance, some models suggest that geographic range limits may be a consequence of severe migration loads that lead to extirpation of geographically peripheral populations (Garcia-Ramos and Kirkpatrick 1997; Holt and Gomulkiewicz 1997; Kirkpatrick and Barton 1997; Ronce and Kirkpatrick 2001; Bridle and Vines 2007). More generally,

immigration may impose a fitness load on recipient populations that can reduce their productivity or stability. Migration load may be of particular concern to conservation biologists. One of the major goals in conservation biology is to maintain connections between habitat patches (Harrison 1994), so as to maintain populations in the face of demographic stochasticity. Unfortunately, this goal may have detrimental side effects in certain settings, as immigration may also have a negative effect on mean fitness, and thus possibly the viability, of recipient populations (Storfer 1999). Understanding the magnitude of such migration load, and factors that influence its severity, could inform conservation policy.

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## Supplementary Material

The following supplementary material is available for this article:

**Appendix S1.** Raw data concerning census size and the percent of striped individuals from samples collected early and late in each generation.

This material is available as part of the online article from:

<http://www.blackwell-synergy.com/doi/abs/10.1111/j.1558-5646.2007.00173.x>

(This link will take you to the article abstract).

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