

Reproductive isolation caused by visual predation on migrants between divergent environments

Patrik Nosil

Department of Biosciences, Behavioural Ecology Research Group, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada (pnosila@sfu.ca)

In theory, natural selection can drive adaptation within species while simultaneously promoting the formation of new species by causing the evolution of reproductive isolation. Cryptic coloration is widespread in nature and is generally considered to be a clear visual example of adaptation. I provide evidence that population divergence in cryptic coloration can also cause reproductive isolation. First, a manipulative field experiment using walking-stick insects demonstrates that the relative survival of different colour-pattern morphs depends on the host-plant species on which they are resting, but only in the presence of avian predation. Second, natural populations adapted to different host plants have diverged in colour-pattern-morph frequencies such that between-host migrants are more likely to be the locally less-cryptic morph than are residents. Collectively, these data indicate that high rates of visual predation on less-cryptic migrants are likely to reduce encounters, and thus interbreeding, between host-associated populations. Comparison with previous estimates of sexual isolation reveals that the contribution of selection against between-host migrants to total premating isolation is as strong as, or stronger than, that of sexual isolation. These findings highlight the potential role of natural selection against migrants between divergent environments in the formation of new species.

Keywords: crypsis; field experiment; natural selection; speciation; *Timema*; walking-stick insects

1. INTRODUCTION

Classical work on speciation focused on the circumstances under which new species are formed, with a particular emphasis on the likelihood and frequency of speciation when populations are geographically separated (allopatry) versus in geographical contact (parapatry or sympatry) (Mayr 1942, 1963). Recent years have seen a resurgence of interest in the exact mechanisms of speciation. In particular, much work has concerned the potential role of adaptive divergence in the evolution of reproductive isolation (Hendry *et al.* 2000; Rundle *et al.* 2000; Schluter 2000 for a review; Jiggins *et al.* 2001; Funk *et al.* 2002; Nosil *et al.* 2002). Most such studies of 'ecological speciation' have quantified reproductive isolation caused by divergent mate preferences (sexual isolation), divergent habitat preferences or ecological selection against hybrids.

I consider a less-recognized, yet general, form of reproductive isolation: reduced survival of between-population migrants (see also Mallet & Barton 1989; Funk 1998; Hendry *et al.* 2000; Via *et al.* 2000; Hendry 2004). This process is often not considered as a form of reproductive isolation but does result in partial reproductive isolation (i.e. reduced gene flow caused by a non-geographical barrier) when it reduces encounters, and thus interbreeding, between individuals from different populations. Reduced migrant fitness may commonly facilitate speciation because it: (i) directly reduces gene flow between populations whenever local adaptation occurs; (ii) can result in partial reproductive isolation without a genetic correlation between genes for local adaptation and genes for mate preference; (iii) imposes selection for further forms of reproductive isolation, such as increased preferences for local habitats and mates (Via *et al.* 2000); and (iv) causes reductions in gene flow, which will increase the

efficacy of selection (Hendry *et al.* 2001). Existing examples of natural selection against migrants are convincing, and come from diverse taxa (Mallet & Barton 1989; Via *et al.* 2000; Schluter 2000 for a review; Vamosi & Schluter 2002), but are usually not framed in terms of the contribution of this process to reductions in gene flow between diverging taxa (i.e. reproductive isolation, but see Mallet & Barton (1989), Funk (1998), Hendry *et al.* (2000) and Via *et al.* (2000)).

Cryptic coloration is extremely common in nature and is usually considered to be a clear visual example of adaptation within species (Cott 1940; Kettlewell 1973). In fact, industrial melanism in the peppered moth is perhaps the most commonly used example of adaptation via natural selection (Kettlewell 1973). However, population divergence in cryptic coloration can also promote speciation whenever high rates of predation on less-cryptic migrants reduce encounters, and thus interbreeding, between individuals from different populations. Despite this intuitive link between crypsis and reproductive isolation, and despite the popularity of crypsis as an example of adaptation within species, its potential role in speciation has received little attention (but see Tauber & Tauber 1977; Endler & Houde 1995).

Timema are wingless phytophagous insects that are distributed throughout western North America (Vickery 1993). Selection against less-cryptic migrants is likely to have played a role in the diversification of the genus *Timema* because host-specific divergence in colour pattern between closely related sympatric and parapatric species is common (Crespi & Sandoval 2000). *Timema cristinae* exhibits genetically determined colour-pattern morphs (Sandoval 1993), with an unstriped morph being more common on the host plant *Ceanothus spinosus* (mean frequency of 81%) and a striped morph being more common on *Adenostoma*

fasciculatum (mean frequency of 72%) (Sandoval 1994a; Nosil *et al.* 2002). Controlled predation trials using wild scrub-jays and captive lizards have demonstrated that each of these morphs is most cryptic on the plant on which it is more common (Sandoval 1994b). Dominance of the unstriped morph is incomplete (Sandoval 1993), and thus a third, intermediate, morph (bearing a faint stripe) is also found at low (less than 5%) frequency in some populations. Local morph frequencies are temporally stable and geographical variation indicates that they are determined by a gene flow–selection balance between host-plant patches exhibiting the different selective regimes (Sandoval 1994a). However, previous work on selection for crypsis in *T. cristinae* has not estimated the relative survival of each morph on each host plant in nature, and fitness in the absence of predation is unknown, precluding estimates of the magnitude of reproductive isolation caused by visual predation on between-host migrants.

Two criteria are required for crypsis to contribute to reproductive isolation between populations of *T. cristinae* adapted to the use of different host-plant species. First, the survivals of different colour-pattern morphs in natural populations must depend on the host-plant species on which they are resting, but only in the presence of visual predation. Second, for immigrants to exhibit reduced fitness relative to residents, populations using different hosts must have diverged in colour-pattern-morph frequencies such that immigrants are more likely to be the locally non-matching morph than are residents. First, I use a manipulative field experiment and data on morph frequencies in natural populations of walking-stick insects to test whether these conditions are fulfilled. Second, I consider the effects of relative population sizes on the strength of selection against migrants. Finally, previous studies of reproductive isolation in this system have examined only sexual isolation, and thus I compare the relative contributions of selection against between-host migrants and sexual isolation to total premating isolation (data on sexual isolation are taken from Nosil *et al.* 2002, 2003).

2. MATERIAL AND METHODS

(a) *Mark–recapture experiment*

Individual *T. cristinae* were collected using sweep nets in the Santa Ynez Mountains, California, USA, in February 2003 (latitude 34°30.55' N, longitude 120°4.17' W). Animals were captured in the first instar on both *C. spinosus* and *A. fasciculatum*, and reared to maturity in the laboratory on *C. spinosus*. Prior to release, each walking-stick insect was scored for colour pattern (unstriped, striped, intermediate) on two separate days, photographed with a digital camera and individually marked with a fine-tipped permanent marker on the abdomen, such that the mark would not be visible when the insect was resting in its natural position on the host plant. Colour pattern was scored by one individual (P. Nosil) and was highly repeatable (380 out of 384 individuals were given identical scores on both occasions). All individuals were marked and released within 5 days of attaining maturity.

I used a replicated random-blocks design with four treatment levels (*Ceanothus* versus *Adenostoma* with avian predators present versus absent, one bush per replicate). Avian predators were excluded using chicken-wire enclosures (3 cm mesh). Each of the four treatments was represented twice within each of two study

sites with 24 individuals released onto each bush ($n = 96$ individuals for each of the four treatments; each bush previously cleared of all *Timema*; area near latitude 34°30.90' N, longitude 119°48.01' W). Upon release, sex ratios were equal and morph frequencies were similar among bushes. Sample bushes were separated from all other suitable host plants by a minimum distance of 5 m (12 m is a typical maximum per-generation dispersal distance; Sandoval 1993). Availability of mature test animals necessitated starting the experiments at the two sites on slightly different dates (26 March 2003 for site 1 and 14 April 2003 for site 2).

Recapture surveys were conducted 3, 10, 17 and 24 days after release (no individuals were recaptured during the final recapture session). For each sample bush, I placed a white cotton sheet underneath the bush, visually inspected the bush for walking-stick insects and then shook each branch such that any undetected insects would fall onto the sheet. I recorded which insects were recaptured and then each specimen was released at the site of capture. A recapture session was considered to be complete when no walking-stick insects were found after 15 min of shaking the branches of a particular bush. On the initial release date and on each subsequent recapture date, I identified which birds were present at the study sites. At both sites, the following insectivorous bird species were observed foraging on or near the experimental bushes: *Aphelocoma californica*, *Sayornis saya*, *Pipilo maculatus*, *Pipilo crissalis*, *Sturnella neglecta*, *Psaltiriparus minimus* and *Turdus migratorius*.

The probability of post-release recapture was analysed in a four-factor logistic regression model that included sex, treatment (C-pred, *Ceanothus* with predators; A-pred, *Adenostoma* with predators; C-nopred, *Ceanothus* without predators; A-nopred, *Adenostoma* without predators), morph (unstriped, intermediate, striped) and bush number (1–4: a 'block' effect), as well as all possible interactions among these factors (adding 'site' as a factor does not influence the results). Treatment- and morph-specific recapture probability, independent of bush and sex, is indicated by a significant interaction between morph and treatment in a reduced regression model derived using backward elimination (the initial model included all factors and interactions but then all terms for which the significance of $-2 \log LR$ was greater than 0.10 were removed).

(b) *Estimating morph-specific survival*

Morph-specific survival probabilities were then estimated using MARK (White & Burnham 1995). For each treatment separately, I began with a fully time-dependent Cormack–Jolly–Seber (CJS) model (Lebreton *et al.* 1992) that included morph, time (one time period for each recapture session) and the interaction between morph and time for both survival and recapture probabilities. In all cases, this full model provided a good fit to the data (goodness of fit tests using χ^2 values or a non-parametric bootstrap approach with 1000 replicates; all $p > 0.10$) and thus represents a reasonable starting point for the analyses (Lebreton *et al.* 1992). Because the full models fit the data well, I then used Akaike information criteria (AIC) (Akaike 1973) and likelihood-ratio (LR) tests (Edwards 1992) to find models that best fit the data. The best-fit model was compared with other models using AIC and LR criteria and was used to estimate survival differences among morphs, where appropriate (best-fit models for each treatment were: C-pred, CJS fully time-independent survival differences among morphs; A-pred, CJS survival differences among morphs dependent on two time periods (1: release to first recapture; 2: all other recapture sessions); C-nopred, CJS time-dependent survival, time-

independent recapture; A-nopred, CJS time-dependent survival, morph-dependent recapture).

In all cases, I corrected for over- or under-dispersion in the data using estimates of the variance inflation factor (\hat{c}) (Lebreton *et al.* 1992), derived by comparing the deviance of the full model with the mean deviance of 1000 simulated datasets (using non-parametric bootstrap of the original dataset). Analyses conducted using a constant \hat{c} of 1.0 gave results congruent with analyses adjusting for lack of fit (results not shown).

(c) *Inferring migrant and resident survival*

The survival of between-population migrants versus residents was inferred by extrapolating from the field experiment to 15 different pairs of natural populations. A 'population' of walking-stick insects is defined as the insects collected within a homogeneous patch of a single host plant (validated by previous mark-recapture and molecular data indicating that most individuals in a patch are residents; Sandoval 1993; Nosil *et al.* 2003; Nosil & Crespi 2004). 'Residents' are defined as the walking-stick insects captured within a focal population and 'migrant' refers to potential migrants, which are walking-stick insects captured outside the focal population and within one or more of the other study populations (usually a neighbouring population using the alternate host). 'Parapatric' insect populations are in contact with a population of insects adapted to the alternative host, whereas 'allopatric' populations are separated from all other populations adapted to the alternative host by distances greater than 50 times the 12 m per-generation gene-flow distance (Sandoval 1993).

Colour-pattern-morph frequencies were surveyed in 15 pairs of populations by catching walking-stick insects with sweep nets during February–June in 2000–2003 ($n = 5229$; 12 parapatric pairs comprised one population using each host plant, two allopatric pairs used the same host plant and one allopatric pair used different host plants). Chi-square tests were used to test for differences among paired populations in morph frequencies.

Selection against between-population migrants was estimated using the following data: (i) morph frequencies of the residents within a focal population (%UR, %IR and %SR = %unstriped, intermediate and striped morphs, respectively, within a focal population); (ii) morph frequencies in another population from which potential migrants originated (%UM, %IM and %SM = %unstriped, intermediate and striped morphs, respectively, within the population that migrants originate from); and (iii) the mean relative survival of each colour-pattern morph in the mark-recapture experiment (using the treatments where predators were present; SUC, SIC and SSC = relative survival of unstriped, intermediate and striped morphs, respectively, on *Ceanothus* and SUA, SIA and SSA = relative survival of unstriped, intermediate and striped morphs, respectively, on *Adenostoma*).

This calculation entailed four steps. First, the relative survival of each morph on each host plant was calculated by assigning a value of one to the morph with the highest survival on each host plant and then scaling the survivals of the other two morphs appropriately (e.g. $1/\text{absolute survival of the morph with highest survival}$ is the factor by which the survival of the other two morphs is multiplied). Second, the frequency of each morph within a population was multiplied by that morph's relative survival on a particular host plant and the values from each of the three morphs summed to yield the mean relative survival of individuals from a given population on a given host plant. For example, the mean survival of residents of a *Ceanothus* population would be calculated as $(\%UR \times SUC)$

$+ (\%IR \times SIC) + (\%SR \times SSC)$ and the mean survival of migrants from another population would be calculated as $(\%UM \times SUC) + (\%IM \times SIC) + (\%SM \times SSC)$. The same calculations were then carried out for the other population in a pairwise comparison, this time reversing the resident and migrant designations (notably when *Adenostoma* populations were considered SUA, SIA and SSA values were used). Third, the strength of selection against migrants within each population was estimated as $(1 - \text{mean survival of migrants}/\text{mean survival of residents})$. Fourth, the strength of selection against migrants for a population pair was estimated by averaging the mean values that were calculated for each single population.

For parapatric populations, I examined the relationship between the magnitude of selection against migrants (averaged across a population pair) and the asymmetry in population size between a pair of adjacent populations. Population size was inferred from the relative area of the host-plant patch used by each population (patch size has been shown to be strongly and positively correlated with population size: $r^2 = 0.63$ and 0.53 for *Ceanothus* and *Adenostoma* patches, respectively; $n = 13$ patches of each host) (Sandoval 1994b).

(d) *Relative contributions of reduced migrant survival and sexual isolation*

I estimated the total premating isolation caused by the combined effects of selection against migrants and sexual isolation, and the relative contribution of each of these two individual components to total isolation (see Ramsey *et al.* 2003 for details of the estimation procedure). In brief, individual components of reproductive isolation specify the magnitude of reproductive isolation caused by a given barrier to gene flow and generally vary from zero to one. The individual contribution of selection against migrants (RI_m) was estimated as $1 - (\text{migrant survival}/\text{resident survival})$ and the individual contribution of sexual isolation (RI_s) as $1 - (\text{heterotypic mating frequency}/\text{homotypic mating frequency})$ (migrant survival was estimated as described in § 2c; mating data are taken from Nosil *et al.* (2002, 2003)). Total reproductive isolation is computed as a multiplicative function of the individual components at sequential stages in the life history, but a given component of reproductive isolation can eliminate only gene flow that has not been eliminated by a previous component. Selection against migrants acts before sexual isolation in the life history and thus the absolute contribution of selection against migrants is $AC_m = RI_m$, the absolute contribution of sexual isolation is $AC_s = RI_s (1 - AC_m)$ and total isolation is $AC_m + AC_s$. The relative contribution of any component is simply the absolute contribution divided by total isolation.

First, using the populations examined in the current and a previous study, I estimated components of reproductive isolation between pairs of populations under three major eco-geographical scenarios: allopatric pairs of populations using the same host-plant species (two pairs from the current study to estimate migrant survival; all mating trials from Nosil *et al.* (2003) where the sexes were from the same population (homotypic matings) or from different allopatric populations (heterotypic matings) using the same host to estimate sexual isolation); allopatric pairs of populations using different host-plant species (single pair from the current study to estimate migrant survival; all mating trials from Nosil *et al.* (2003) where the sexes were from the same population or from different allopatric populations using different hosts to estimate sexual isolation); and parapatric pairs of populations using different host-plant species (morph frequencies from the 12 pairs in the current study

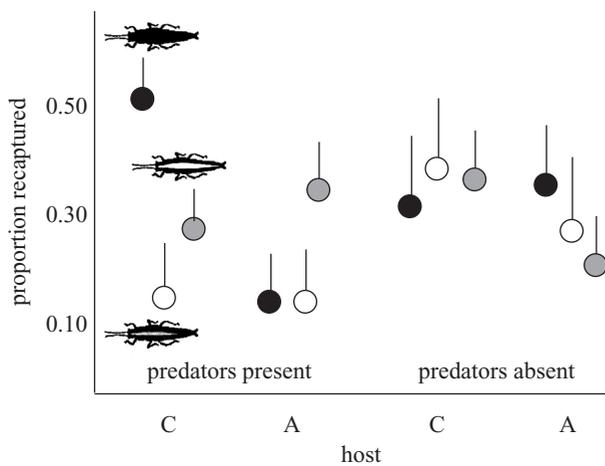


Figure 1. Proportion recaptured (± 1 s.e.) of different colour-pattern morphs of *Timema cristinae*, under four selection regimes (*Ceanothus* (C) and *Adenostoma* (A) as host plants in the presence and absence of avian predation). Black circles, unstriped; white circles, intermediate; grey circles, striped.

pooled to estimate mean migrant survival; all mating trials from Nosil *et al.* (2003) where the sexes were from the same population or from different populations that have an adjacent neighbouring population on the alternative host to estimate sexual isolation).

Second, I estimated levels of reproductive isolation for a range of relative sizes of the neighbouring population using each of the 12 study populations in Nosil *et al.* (2003) in a single comparison (all the populations examined in the current study were examined in Nosil *et al.* (2003) but not vice versa). I calculated the level of premating isolation caused by natural selection against migrants and the level of sexual isolation that each of the 12 focal populations examined in Nosil *et al.* (2003) exhibits against all other study populations from Nosil *et al.* (2003) that use the alternative host plant (individuals within a population were considered to be resident and individuals from all other populations were considered to be migrants; within-population mating trials were considered to be homotypic and all between-population mating trials were considered to be heterotypic). This averaging among populations should not bias the results, although it will decrease the precision of the estimates. I then examined the association between geographical scenario (i.e. the relative size of the neighbouring population, allopatric populations assigned a value of zero) and reproductive isolation using regression analyses. Departures from linearity were detected in some cases and thus partial *F*-tests were used to test whether a model including both a linear term and a quadratic term provided a better fit than a model with only a linear term.

3. RESULTS

(a) *Mark-recapture experiment*

Mark-recapture experiments show that the recapture probability (percentage recaptured) of different colour-pattern morphs is dependent on the host plant and on the presence of avian predators (mean recapture rates for each morph in each treatment are shown in figure 1; morph \times treatment interaction: LR = 14.16, d.f. = 6, $p < 0.05$). Consequently, I used maximum-likelihood techniques that are designed specifically for the analysis

of mark-recapture data (i.e. that independently estimate survival and recapture probabilities) to estimate survival differences among the colour-pattern morphs within each treatment.

(b) *Host-specific survival of colour-pattern morphs*

When avian predators were present, the colour-pattern morphs differed in survival (*Ceanothus*: best model includes a morph survival term, AIC = 108.3, 2.26 times better than the next-best model; LR = 5.95, d.f. = 2, $p < 0.05$; *Adenostoma*: best model includes a morph survival term, AIC = 164.93, 5.88 times better than the next-best model; LR = 7.88, d.f. = 2, $p < 0.05$). The unstriped morph exhibited the highest survival on *Ceanothus* and the striped morph exhibited the highest survival on *Adenostoma* (figure 2). The intermediate morph exhibited survival similar to that of the striped morph (low on *Ceanothus* and high on *Adenostoma*), suggesting that a faint stripe functions similarly to a pronounced stripe. The best-fit model included a time-component on *Adenostoma*, but not on *Ceanothus*, and the results are presented as such in figure 2 (see § 2b for details of model-testing procedures).

Survival differences among colour-pattern morphs disappear when predators are excluded (for both *Ceanothus* and *Adenostoma*, the best model does not include a morph survival term, AIC = 177.95 and 196.03, respectively, 436 and 123 times better, respectively, than the best models that include a morph survival term). Thus survival of different colour-pattern morphs is dependent on host-plant species only in the presence of avian predation. Notably, morph-specific dispersal cannot account for these results as walking-stick insects could disperse from both predator and predator-free treatments, yet morph-specific survival occurred only when predation was present. Collectively, these data demonstrate divergent host-specific selection on colour pattern and indicate that differential visual predation is the agent of selection.

(c) *Estimates of reproductive isolation caused by reduced migrant survival*

Populations of *T. cristinae* using different hosts have diverged in colour-pattern-morph frequencies such that immigrants are more likely to be the locally non-matching morph than are residents (table 1). Thus between-host migrants are likely to exhibit reduced survival relative to residents (s , strength of selection against between-host migrants averaged across the 13 pairs using different hosts = 0.18). This reduced survival of migrants will cause reproductive isolation if it lowers encounter rates, and thus interbreeding, between host-associated populations.

The degree of fitness reduction exhibited by migrants was variable and dependent on the ecological and geographical scenario examined (figure 3). Under a scenario of secondary contact between allopatric populations, different populations using the same host-plant species would exhibit no difference in the fitnesses of migrants and residents (owing to similarities in morph frequencies and in the selective environment; $s = 0.00$). By contrast, the geographically separated populations using different host plants are strongly differentiated in morph

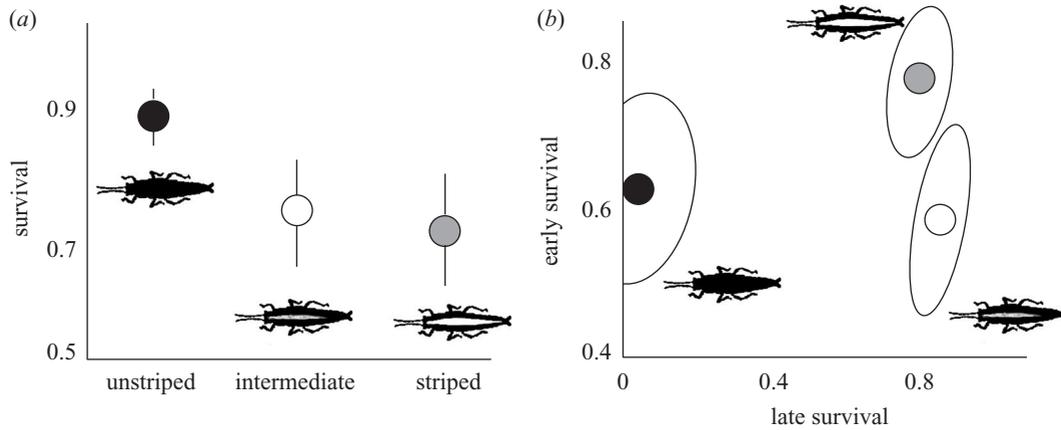


Figure 2. When exposed to visual predation, survival probabilities varied among colour-pattern morphs of *Timema cristinae* on the two different host-plant species ($p < 0.05$ on both hosts). (a) On *Ceanothus*, survival was independent of time, with the unstriped morph exhibiting the highest survival during the study (see § 2b for model-testing procedures). (b) By contrast, in the best-fit model for *Adenostoma* survival was dependent on an interaction between time and morph, with the striped (grey circle) morph exhibiting somewhat higher survival than the unstriped morph early in the experiment (between initial release and the first recapture session) and much higher survival for the remainder of the experiment ('late survival'). When visual predation was excluded, survival did not vary among morphs (results not shown, both $p > 0.25$). Populations using different host plants have diverged in morph frequencies such that each morph is most common on the host plant on which it has the highest survival. Thus, on average, individuals that migrate to the alternative host plant exhibit reduced fitness relative to residents (mean $s = 0.18$). (a) *Ceanothus*: lines show 95% confidence intervals. (b) *Adenostoma*: ellipses show 95% confidence intervals (black circle, unstriped morph; white circle, intermediate morph; grey circle, striped morph).

Table 1. Morph frequencies in the sample populations of *Timema cristinae*.

(Numbers of each morph found within 30 populations are shown (U, unstriped; S, striped; I, intermediate). Populations were paired such that 12 pairs of populations were adjacent parapatric pairs using different hosts (pairs 1–12) and three pairs were allopatric (pair 13 consists of one population using each host, and pairs 14 and 15 consist of two allopatric populations each using *Ceanothus*). For parapatric pairs, %C refers to the total area of the two populations that is occupied by *Ceanothus*. Also shown are χ^2 values testing whether morph frequencies differ between paired populations and overall.)

	<i>Ceanothus</i> ^a population			<i>Adenostoma</i> ^a population				χ^2
	U	S	I	U	S	I	%C	
parapatric pairs								
1	28	43	6	101	485	14	34	26.01***
2	5	6	2	21	133	8	39	9.52**
3	447	44	0	213	79	9	94	59.78***
4	10	10	0	70	322	7	8	13.12**
5	52	12	3	201	166	13	67	15.83***
6	158	97	80	86	175	81	20	43.55***
7	7	3	3	3	4	2	5	1.26
8	12	7	4	10	47	9	65	14.30***
9	30	21	0	11	5	1	12	3.54
10	27	68	0	3	27	0	5	4.24*
11	30	82	3	6	51	1	1	7.09*
12	2	12	4	5	56	10	2	1.19
pooled	808	405	105	730	1550	155		387.51***
allopatric pairs								
13	423	5	1	69	337	4	N/A	576.87***
14	382	90	14	45	21	3	N/A	6.12*
15	35	0	0	43	2	2	N/A	3.13

^a This host designation applies only to parapatric pairs in the table.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

frequencies and thus would exhibit highly reduced levels of migrant fitness ($s = 0.53$). Among parapatric populations using different host plants, selection against migrants, taken as the mean of the two populations, weak-

ens as asymmetry in population size between paired populations increases (range of $s = 0.02$ – 0.30 , mean $s = 0.15$, s.d. = 0.08 ; $r = 0.51$, $p < 0.05$; $n = 12$ paired populations, Spearman's rank correlation).

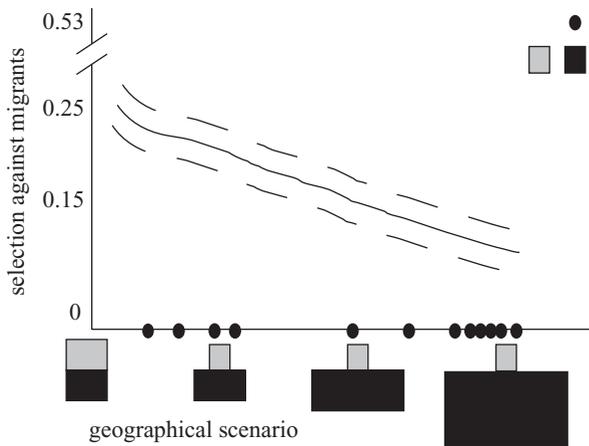


Figure 3. The strength of selection against between-host migrants for 12 different pairs of adjacent populations, under various geographical scenarios (x -axis). Each population pair consists of one population that uses *Ceanothus* as the host plant and one that uses *Adenostoma* (total $n = 3753$; see table 1 for population-specific sample sizes). The values on the y -axis represent the strength of selection against migrants ($1 - \text{migrant survival/resident survival}$) averaged across the two populations in each pairwise comparison. Reduced fitness of migrants is greatest when population sizes are similar (i.e. when asymmetric gene flow is least likely to homogenize morph frequencies). The curve was estimated using the non-parametric cubic spline (dashed lines show standard errors from 1000 bootstrap replicates) (Schluter 1988). The geographical scenario for each parapatric pair of populations is denoted by a black circle on the x -axis. Also shown for comparative purposes is the strength of selection against migrants under a scenario of secondary contact between two allopatric populations using different host plants (top right).

(d) *Relative contributions of reduced migrant survival and sexual isolation*

In *T. cristinae*, total premating isolation is non-existent for allopatric pairs using the same host, strongest for allopatric pairs using different hosts and intermediate for parapatric pairs using different hosts (figure 4a). Selection against between-host migrants can contribute to total reproductive isolation between populations using different hosts as strongly as does sexual isolation (relative contributions to total reproductive isolation of 0.68 versus 0.32, 0.90 versus 0.10 and 0.38 versus 0.62 for all, allopatric only and parapatric only populations, respectively).

Consideration of the potential for gene flow into each of the 12 study populations examined in Nosil *et al.* (2003) revealed a more refined picture of the evolution of reproductive isolation (figure 4b). The individual component of reproductive isolation caused by selection against migrants was highest between allopatric populations and declined with increasing migration into the focal study population (linear regression: $r^2 = 0.81$, slope of the regression $B = -0.42$, s.e. = 0.07, $p < 0.001$; F -change between linear and quadratic model = 1.91, $p = 0.20$). By contrast, the individual component of reproductive isolation caused by sexual isolation was weak between allopatric populations, increased until the size of the study population was similar to that of its neighbouring population using the alternative host plant and decreased as the neighbouring population became relatively larger (linear regression: $r^2 = 0.05$, $B = -0.05$, s.e. = 0.07, $p = 0.48$; quadratic regression:

$r^2 = 0.44$, $B = -0.53$, s.e. = 0.21, $p < 0.05$; F -change between linear and quadratic model = 6.30, $p < 0.05$; as reported in Nosil *et al.* (2003)). Thus total premating isolation was high across a wide range of geographical scenarios, with selection against migrants contributing strongly to total isolation when the size of the population adjacent to the study population was small (or when the study population was allopatric) and sexual isolation contributing most strongly to total isolation when the sizes of the study and the neighbouring populations were similar (figure 4b).

4. DISCUSSION

Natural selection against migrants between divergent environments can cause reproductive isolation by reducing encounters, and thus interbreeding, between individuals from populations exhibiting divergent local adaptations (Mallet & Barton 1989; Funk 1998; Via *et al.* 2000; Hendry 2004). In this study, a manipulative field experiment and data on morph frequencies in natural populations were used to infer the survivals of migrant and resident walking-stick insects under various geographical and ecological scenarios. The results show that migrants between populations adapted to the use of different host-plant species (i.e. divergent environments) are likely to exhibit increased predation rates and thus reduced survival relative to residents. This process will cause reproductive isolation between populations using different host plants when it reduces encounters, and thus interbreeding, between host-associated populations. These findings are of particular interest because the agent of selection (differential visual predation) is well understood and differences in survival among morphs disappeared when it was excluded.

The survival of between-population migrants relative to residents was inferred by extrapolating from the field experiment to 15 different pairs of natural populations. Thus the results may slightly overestimate selection against migrants as they do not account for the reduced predation pressure on less-cryptic prey that can arise from density- or frequency-dependent predation (Bond & Kamil 2002). Nonetheless, geographical variation in morph frequencies is consistent with increased survival of the more common morph on each host-plant species in nature (Sandoval 1994b). Furthermore, lower relative survival of the less-cryptic morph in the mark-recapture experiment was independent of time on *Ceanothus* and was strongest in the latter stages of the experiment on *Adenostoma*, providing evidence that the less-cryptic morph will indeed be subject to increased predation even as it becomes increasingly rare.

My results are consistent with previous studies of host-associated pea aphids (Via *et al.* 2000) and leaf beetles (Funk 1998), mimetic *Heliconius* butterflies (Mallet & Barton 1989) and sympatric sticklebacks (Schluter 1995; Vamosi & Schluter 2002), which have also explicitly noted that natural selection against migrants between divergent environments is likely to reduce gene flow between diverging taxa. In particular, the results compliment the work on *Heliconius*, where migrants suffer increased rates of visual predation owing to a loss of mimicry rather than crypsis. However, reduced migrant fitness (i.e. local adaptation) is widespread in nature and thus many other potential

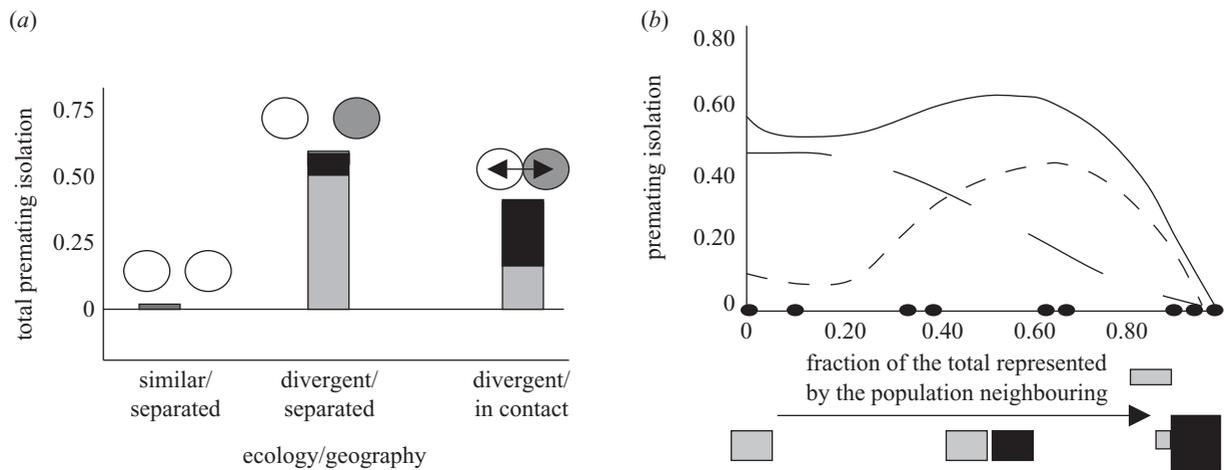


Figure 4. Components of reproductive isolation under different ecological and geographical scenarios. (a) Absolute contributions of natural selection against migrants and sexual isolation to total pre-mating isolation for populations of walking-stick insects under three different ecological and geographical scenarios. The relative contribution of each component is simply its absolute contribution divided by total isolation. Black sections of bars represent sexual isolation and grey sections of bars represent migrant survival. (b) For the 12 populations also examined in Nosil *et al.* (2003), the average magnitude of reproductive isolation that a study population exhibits against all other populations using the alternative host (total isolation and individual components are shown). The x-axis represents the fraction of the total represented by the neighbouring population (study population, grey box; neighbouring population, black box). Similar levels of total pre-mating isolation are observed across a range of geographical scenarios (i.e. roughly until the size of the neighbouring population exceeds that of the study population), but arise via different individual components of reproductive isolation. Solid line, total pre-mating isolation; short-dashed line, sexual isolation; long-dashed line, reduced migrant survival.

examples exist (see Schluter (2000) for a review). Notably, reproductive isolation caused by selection against migrants is independent of actual mating preferences, which may increase or decrease total levels of reproductive isolation (if foreign males are discriminated against or preferred as mates, respectively).

The current study expands on the previous work on reproductive isolation caused by selection against migrants by explicitly examining the role of relative population sizes (i.e. population demography). Colour-pattern-morph frequencies in populations of *T. cristinae* are determined by a balance between gene flow and selection such that morph frequencies are strongly differentiated between allopatric populations using different host-plant species, moderately differentiated between adjacent populations of similar size that use different hosts, weakly differentiated between adjacent populations of unequal size that use different hosts, and almost identical in populations using the same host plant (Sandoval 1994a). Thus selection against migrants, taken as the mean of the two populations, weakens as asymmetry in population size between paired populations increases. These data indicate that selection against migrants is most likely to promote speciation when secondary contact between allopatric populations is accompanied by: (i) ecological divergence; and (ii) equality in the sizes of the diverging populations (i.e. when asymmetric gene flow is least likely to erode population differentiation).

Although natural selection against migrants may commonly reduce gene flow between populations, such reproductive isolation is environment dependent and incomplete. Nonetheless, in *T. cristinae* selection against between-host migrants can contribute to total reproductive isolation as strongly as does sexual isolation. In addition, there are both theoretical and empirical data

indicating that selection against migrants can facilitate the evolution of further forms of reproductive isolation. For example, the initial reduction in gene flow caused by selection against migrants may promote the evolution of genetic correlations between local adaptation and mating preferences (Servedio & Kirkpatrick 1997) and can increase the efficacy of selection for local adaptation (Hendry *et al.* 2001). The exact importance of selection against migrants in speciation might depend on what stage of the process is examined. For example, anti-migrant selection might be particularly important in the early stages of speciation, as it can evolve rapidly whenever new environments are colonized (Hendry 2004), but may play a more minor role in the latter stages of speciation, when sexual isolation or postmating incompatibilities act as strong barriers to gene flow.

Natural selection against migrants can also promote speciation by imposing selection for increased efficiency of habitat choice and increased mating discrimination against between-population migrants (Via *et al.* 2000), a process analogous to reinforcement (Dobzhansky 1951). In *T. cristinae*, the costs of mating with locally less-cryptic migrants are twofold: (i) the male rides on the back of the female during the mating period and thus females that mate with less-cryptic males are likely to suffer reduced individual survival during mating; and (ii) females that mate with less-cryptic males will produce a higher frequency of less-cryptic offspring than females that mate with resident males (Sandoval 1993). Sexual isolation between populations of *T. cristinae* has apparently evolved in parallel with divergence in host-plant use and is greater in geographical regions where between-host migration occurs than in regions where geographically separated populations do not exchange migrants (Nosil *et al.* 2002, 2003). These data suggest that the costs of mating with

migrants have played a role in driving the evolution of sexual isolation. However, between-population mate choice is not based on colour pattern (Nosil *et al.* 2002) and thus reproductive isolation caused by selection against migrants and reproductive isolation resulting from mate preferences are not based on the same trait(s).

The results of this study have broad implications for understanding both adaptation and speciation as they illustrate how divergent selection for local adaptation in general, and cryptic coloration in particular, can play a role in the speciation process. Moreover, they highlight the potential roles of selection for crypsis and visual predation in the origin of species and thus provide some of the only evidence for the general role of predation in adaptive radiation (Schluter 2000).

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