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Climate affects geographic variation in host-plant but not mating preferences of *Timema cristinae* stick-insect populations

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ABSTRACT

Background: The behaviour of individuals within a species often varies geographically and this can affect reproductive isolation between populations. Published data show that natural selection stemming from both host-plant use and reinforcement affect reproductive isolation in the stick insect *Timema cristinae*, by generating heritable population differences in feeding and mating preferences (i.e. habitat and sexual reproductive isolation, respectively). Genomic divergence in *T. cristinae* is correlated with climate, but the effects of climate on feeding and mating preferences have yet to be tested.

Questions: How do multiple sources of natural selection combine, interact or counteract one another to affect geographic variation in *T. cristinae* behaviour? How does this affect reproductive isolation between populations?

Organisms: Twelve populations of *T. cristinae*. Each is found on one of two host plants (*Ceanothus* or *Adenostoma*) and populations also differ in local climatic conditions. Eight of these populations undergo maladaptive hybridization with an adjacent population and thus exhibit the potential for reinforcement. But the other four populations are geographically isolated.

Methods: We combine published data with new data and analyses on climatic variability among populations. We employ univariate and multiple regression analyses to examine the relationship between mean population-level host-plant feeding and mating preferences and three factors: host-plant use, reinforcement, and climate.

Results and conclusions: Climate was correlated with population-level host preferences: mean preference for the nutritionally superior host (i.e. *Ceanothus*) increased among populations with increasing temperature and aridity. This pattern occurred for populations on both hosts and thus climate neither increased nor decreased habitat isolation between populations on different hosts. We propose selection to prefer *Ceanothus* increases under hotter and drier climatic conditions and does so for populations on both hosts. Climate was not correlated with population-level mating preferences because sexual isolation is largely driven by reinforcement between adjacent populations that are most similar in climate. Thus, host-plant use, reinforcement, and climate, coupled with patterns of gene flow across the landscape, combine to determine geographic variation in the behaviour of *T. cristinae*.

Keywords: climate, gene flow, genomic divergence, host-plant adaptation, reinforcement, speciation, temperature.

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INTRODUCTION

Speciation can be a complex process involving multiple sources of selection (Coyne and Orr, 2004; Schluter, 2009; Nosil, 2012). If these different sources of selection combine to increase the total strength of divergent selection experienced by populations, speciation can be promoted (Nosil *et al.*, 2009). However, different sources of selection might also counteract one another and combine with homogenizing gene flow to constrain population divergence (Bolnick and Doebeli, 2003; Sandoval and Nosil, 2005; Bolnick and Fitzpatrick, 2007; Bolnick and Nosil, 2007; Hendry *et al.*, 2009). In addition to multiple sources of selection, multiple reproductive barriers appear to often be involved in speciation (Nosil *et al.*, 2005; Lowry *et al.*, 2008; Matsubayashi and Katakura, 2009; Schluter, 2009; Matsubayashi *et al.*, 2010; Nosil, 2012), and these may or may not respond in parallel manners to different sources of natural selection. Finally, the sources of selection and the forms of reproductive isolation that evolve due to them might vary across a species range, resulting in widespread geographic variation in the degree and types of reproductive isolation between populations (Endler, 1977; Thompson, 2005; Gabor *et al.*, 2013; Gerhardt, 2013; Pröhl *et al.*, 2013). Joint consideration of these factors will likely increase our understanding of when and how speciation is promoted versus constrained. Along these lines, we examine here geographic variation in two forms of behavioural reproductive isolation among 12 host-associated populations of stick insects subject to a suite of potential sources of divergent selection.

STUDY SYSTEM AND PAST WORK

The study species, *Timema cristinae*, is a wingless, herbivorous insect which is endemic to southern California and which has evolved partially reproductively isolated ‘ecotypes’ adapted to different host-plant species: *Adenostoma fasciculatum* (Rosaceae) and *Ceanothus spinosus* (Rhamnaceae) (for a review, see Nosil, 2007) (Fig. 1). As in past work, ecotypes are defined by the host they are found upon and a population is defined as all the *T. cristinae* within a patch of a single host-plant species. Populations are found under two main geographic arrangements: adjacent patches of the two hosts (‘parapatry’) and patches that are geographically separated from populations on the alternative host (‘allopatry’). Thus, populations vary from one another in several potential sources of selection, including host-plant use, reinforcement, and climatic conditions, as well as in the potential for between-host gene flow. We tested how these factors might affect host-plant feeding and mating preferences, which can contribute to habitat and sexual isolation respectively. Notably, data from genetic crosses as well as common-garden and reciprocal rearing experiments indicate differences among populations in both forms of preference are heritable, although further work on maternal effects is warranted (Nosil *et al.*, 2003, 2006a, 2006b; Nosil, 2007). We outline below in more detail what is known in this system and the new data and hypotheses addressed here.

Host-plant adaptation

Divergent host adaptation occurs in *T. cristinae* and promotes the evolution of reproductive isolation (i.e. ecological speciation) (Nosil, 2007, 2012). For example, ecotypes on different hosts differ in a suite of morphological traits, including colour, colour pattern, size, and shape. Crossing and genome-wide association mapping studies have shown that these differences are heritable (Sandoval, 1993, 1994b; Comeault *et al.*, submitted). Manipulative field experiments have

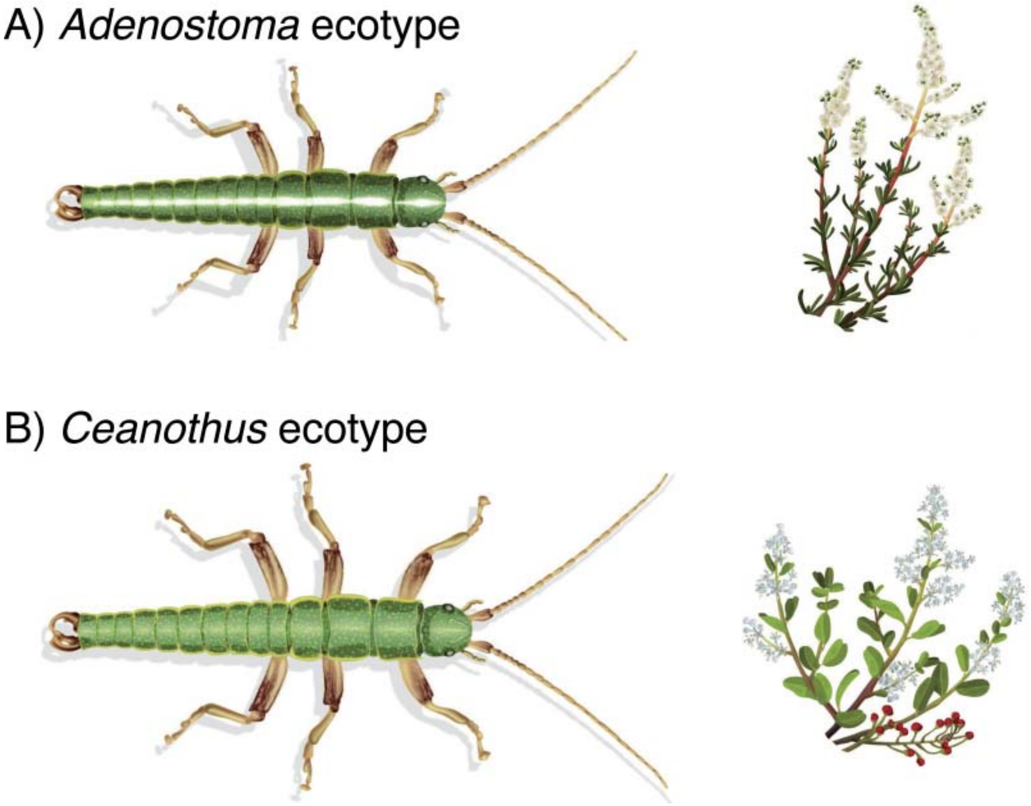

Fig. 1. Illustration of the *T. cristinae* study system, with male specimens of each insect ecotype shown on the left and drawings of the host plants that they are adapted to on the right. Illustrations courtesy of Rosa Ribas.

demonstrated that they evolved due to divergent selection between hosts, driven by selection for crypsis from predators (Sandoval, 1994a, 1994b; Nosil, 2004; Sandoval and Nosil, 2005; Nosil and Crespi, 2006). These morphological differences result in strong selection against between-host migrants and hybrids, generating ecologically based reproductive isolation. In addition, pairs of populations on different host plants exhibit greater divergence in host and mating preferences than pairs of populations in different localities but on the same host plant (Nosil *et al.*, 2002, 2003). Thus, these forms of reproductive isolation have evolved, at least in part, as a by-product of divergent adaptation (see also Langerhans and Makowicz, 2013). However, both habitat and sexual isolation are far from complete. The evolution of divergent host preferences is likely constrained by the fact that in the absence of predators, one host appears universally nutritionally superior to the other, with ecotypes of both hosts exhibiting higher fecundity on *Ceanothus* (Sandoval and Nosil, 2005). Thus, although viability selection from predators selects for divergent host preferences between populations on different hosts, fecundity selection favours preference for *Ceanothus* for both ecotypes.

Gene flow

Gene flow between populations is often a homogenizing force that prevents or constrains population divergence (Endler, 1973; Crespi, 2000; Barton, 2001; Hendry *et al.*, 2001; Hendry and Taylor, 2004; Bolnick and Nosil, 2007; Bolnick *et al.*, 2008; Abbott *et al.*, 2013). Morphological and molecular genetic divergence among populations of *T. cristinae* is constrained by gene flow, as evidenced by previous observational, experimental, and molecular studies (Sandoval, 1994b; Nosil and Crespi, 2004; Bolnick and Nosil, 2007; Nosil, 2007, 2008, 2009; Nosil *et al.*, 2008, 2012a, 2012b). For example, phenotypic and genetic divergence is greater between allopatric population pairs than between parapatric pairs, and levels of local maladaptation within local populations increase with the degree of gene flow into them from populations on the alternative host.

Reinforcement

The selection against migrants and hybrids between hosts described above generates selection on mating preferences for the avoidance of maladaptive hybridization (Gerhardt, 2013). Consistent with the main prediction of reinforcement, parapatric pairs of populations exhibit stronger sexual isolation than allopatric pairs of populations, i.e. reproductive character displacement (Nosil *et al.*, 2003). Furthermore, recent work examining the number of independent axes of trait divergence that underlie sexual isolation in *T. cristinae* revealed that divergence of parapatric populations in mating preferences occurs along a different trait axis than does that of allopatric pairs (Nosil and Hohenlohe, 2012). Finally, genomic studies have shown an excess of exceptionally differentiated high-F_{ST} 'outlier loci' between parapatric populations, relative to that observed between allopatric pairs (Nosil *et al.*, 2012a). Thus, parapatric populations differ from allopatric ones.

The degree of sexual isolation also varies predictably according to levels of gene flow. Theoretical models have demonstrated that high levels of gene flow between populations erode the effects of reinforcing selection (Sanderson, 1989; Servedio and Kirkpatrick, 1997; Cain *et al.*, 1999; Servedio and Noor, 2003). However, gene flow also generates the opportunity for selection against hybridization to occur in the first place. Thus, gene flow can exert a dual effect during reinforcement, as modelled by Kirkpatrick (2000) and exemplified by a quotation in Coyne and Orr's classic book on speciation: 'reinforcement requires some gene flow, but not too much' (Coyne and Orr, 2004, p. 371). The effects of reinforcement are thus predicted to be maximized when gene flow is intermediate, i.e. high enough to allow the evolution of reinforcement, but low enough to prevent homogenization of divergence in mate choice. Few empirical studies have examined the effects of gene flow on reinforcement (for a review, see Servedio and Noor, 2003) and perhaps the clearest example stems from the *T. cristinae* system: the magnitude of female mating discrimination against males from other populations is greatest when gene flow between populations adapted to alternate host plants is intermediate (Fig. 2).

Climate

Evidence emerged only very recently that climatic variability is an important source of natural selection affecting populations of *T. cristinae* (Nosil *et al.*, 2012a, 2012b). The main axis of climatic variability is temperature and aridity, both of which vary with elevation such that higher sites are colder and experience more precipitation (see Tables 1–3 for data from the populations examined in the current study). Most of the evidence for climate-related

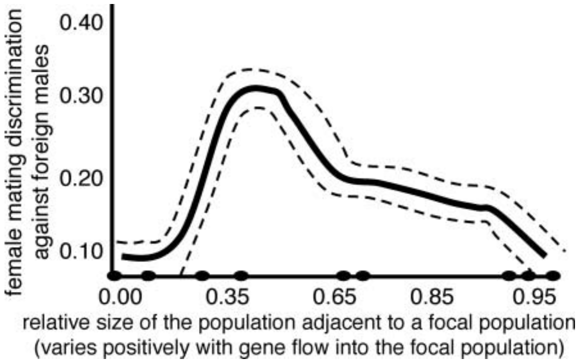

Fig. 2. The effects of gene flow on reinforcement. Among 12 populations of *Timema cristinae*, female mating discrimination against males from other populations is strongest when gene flow into the population, from an adjacent population adapted to a different host-plant species, is intermediate. The x-axis shows the size of the population that is adjacent to a focal study population, relative to the size of the study population itself. This value is positively correlated with the level of gene flow, inferred from molecular data, into the focal study population (relative sizes of populations examined are denoted by circles on the x-axis; note that four allopatric populations had a value of zero on the x-axis). The y-axis is mean copulation frequency of females with males from their own population minus mean copulation frequency of females with foreign males. The curve was estimated using the non-parametric cubic spline with standard errors shown from 1000 bootstrap replicates (Schluter, 1988). Modified from Nosil *et al.* (2003) with permission of the Royal Society of London.

Table 1. Raw bioclimatic data for the study populations, at 0.5 arc minute resolution

	P	HVC	HVA	MA	LA	VPC	VPA	OUTA	PRC	MBOCC	OGA	HA
BIO1	153	151	151	132	132	147	147	147	154	144	132	138
BIO2	126	127	127	124	124	129	129	129	128	128	124	126
BIO3	54	53	53	50	50	53	53	53	54	52	50	51
BIO4	3664	3825	3825	4305	4305	3984	3984	3984	3759	4049	4305	4100
BIO5	274	278	278	270	270	279	279	279	279	277	270	273
BIO6	44	41	41	25	25	37	37	37	43	34	25	30
BIO7	230	237	237	245	245	242	242	242	236	243	245	243
BIO8	113	109	109	85	85	104	104	104	113	100	85	94
BIO9	197	198	198	185	185	196	196	196	200	194	185	189
BIO10	202	203	203	192	192	202	202	202	205	200	192	195
BIO11	109	106	106	84	84	101	101	101	110	98	84	92
BIO12	539	566	566	679	679	593	593	593	519	622	679	650
BIO13	116	122	122	139	139	126	126	126	111	133	139	137
BIO14	0	0	0	0	0	0	0	0	0	0	0	0
BIO15	99	97	97	95	95	97	97	97	96	98	95	96
BIO16	323	338	338	389	389	351	351	351	309	369	389	380
BIO17	2	2	2	3	3	2	2	2	2	2	3	2
BIO18	8	8	8	13	13	10	10	10	8	10	13	11
BIO19	308	323	323	376	376	334	334	334	291	353	376	365

Note: See Table 3 for descriptions of bioclimatic variables. Temperature values have been multiplied by 10 to reduce file sizes, as standard on the WorldClim website

Table 2. Characteristics of the *T. cristinae* populations studied

Code	Host	Geog.	Adjacent size	Latitude	Longitude	PC1	PC2	Hpref	Mpref
P	C	A	0.00	34.477	−119.768	−3.820	−0.506	93	0.10
HVC	C	P	0.66	34.490	−119.786	−2.486	−1.236	79	0.12
HVA	A	P	0.34	34.489	−119.787	−2.486	−1.236	78	0.16
MA	A	P	0.39	34.515	−119.798	5.079	0.710	62	0.41
LA	A	A	0.00	34.509	−119.796	5.079	0.710	63	0.02
VPC	C	A	0.00	34.532	−119.843	−1.035	−1.949	91	−0.05
VPA	A	P	0.94	34.529	−119.843	−1.035	−1.949	84	0.17
OUTA	A	P	0.67	34.532	−119.844	−1.035	−1.949	86	0.13
PRC	C	A	0.00	34.534	−119.858	−4.064	−0.905	93	0.08
MBOCC	C	P	0.95	34.504	−119.805	0.212	−2.044	87	0.19
OGA	C	P	1.00	34.513	−119.796	5.079	0.710	76	−0.08
HA	A	P	0.08	34.503	−119.826	2.296	−0.435	81	0.00

Note: For host designation, C = *Ceanothus* and A = *Adenostoma*. Geog. refers to the geographic arrangement of the population (A = allopatric, P = parapatric). PC1 and PC2 are principal components scores from climatic data. Hpref = host preference. Mpref = mate preference. See main text for details.

Table 3. Loading scores for principal component (PC) axes

	PC1	PC2
BIO1 = annual mean temperature	−0.27	−0.11
BIO2 = mean diurnal range (mean of monthly (max. temp. – min. temp.))	−0.11	−0.43
BIO3 = isothermality (BIO2/BIO7) (× 100)	−0.26	0.15
BIO4 = temperature seasonality (standard deviation × 100)	0.25	−0.23
BIO5 = max. temperature of warmest month	−0.05	−0.47
BIO6 = min. temperature of coldest month	−0.28	0.04
BIO7 = temperature annual range (BIO5 – BIO6)	0.19	−0.34
BIO8 = mean temperature of wettest quarter	−0.28	−0.01
BIO9 = mean temperature of driest quarter	−0.20	−0.32
BIO10 = mean temperature of warmest quarter	−0.19	−0.34
BIO11 = mean temperature of coldest quarter	−0.28	0.01
BIO12 = annual precipitation	0.27	−0.08
BIO13 = precipitation of wettest month	0.26	−0.16
BIO15 = precipitation seasonality (coefficient of variation)	−0.14	−0.33
BIO16 = precipitation of wettest quarter	0.27	−0.13
BIO17 = precipitation of driest quarter	0.22	0.09
BIO18 = precipitation of warmest quarter	0.27	0.02
BIO19 = precipitation of coldest quarter	0.27	−0.11

Note: PC1 and PC2 explained 70% and 24% of the variance in climatic conditions among populations respectively. BIO14 (precipitation of driest month) is excluded due to being non-variable across our study sites.

natural selection stems from genomic data, although some of these data are directly experimental. Observational population genomic scans using tens of thousands of single nucleotide polymorphisms revealed allele frequency clines and exceptionally strong genetic divergence (high-F_{ST} 'outlier loci') correlated with climatic conditions (Nosil *et al.*, 2012a). Analyses of genome-wide patterns of introgression also revealed evidence for selection against alleles associated with divergent climatic conditions within admixed hybrid populations (Nosil *et al.*, 2012b). Finally, field transplant experiments revealed: (1) greater mortality when *T. cristinae* were transplanted to more divergent climatic conditions (Nosil *et al.*, 2012a), and (2) rapid allele frequency changes across the genome associated with transplantation to novel climatic environments (P. Nosil and Z. Gompert, unpublished).

In contrast to accumulating evidence that climate affects genomic divergence, nothing is yet known concerning whether climate affects host or mating preferences, an issue we examine here. Since climatic variables such as temperature or precipitation are known in other systems to have a profound influence on mate choice (Cockburn *et al.*, 2008; Cornwallis and Uller, 2010; Safran *et al.*, 2010) as well as host preferences (Leather *et al.*, 1994; Sotka and Giddens, 2009; Ipekdal and Caglar, 2012), it is reasonable to predict such effects might occur also in *T. cristinae*, especially given the evidence for climate-related selection on the genome.

METHODS

Data on host and mating preference

The data on behavioural reproductive isolation considered here are those presented in Nosil and Yukilevich (2008) and population means are given in Table 2. The raw data on host preference stem from Nosil *et al.* (2006b), who used dichotomous choice trials to determine whether individuals chose to rest on *Ceanothus* or *Adenostoma*. Here, our index of mean population host preference is the proportion of individuals choosing *Ceanothus* over *Adenostoma* in these trials ('host preference' hereafter). The raw data on mating preference stem from Nosil *et al.* (2003), who used no-choice mating trials to measure whether a male and female copulated with one another within a one-hour period. Here, our index of mean population mating discrimination against individuals from other populations was calculated as follows: the mean copulation frequency of females with males from their own population subtracted from the mean copulation frequency of females with foreign males from other populations ('mate preference' hereafter). This averaging among populations provides a general measure of the degree to which a focal population is sexually isolated from other populations (Nosil and Yukilevich, 2008). In some cases, we examined the degree of sexual reproductive isolation between specific pairs of populations. When doing so we used an index of assortative mating, the I_{PSI} index, for which values of zero indicate random mating and one indicates complete sexual isolation (Carvajal-Rodriguez and Rolan-Alvarez, 2006).

Climate data

We retrieved bioclimatic data from the WorldClim website (http://www.worldclim.org/) (Hijmans *et al.*, 2005) at the highest resolution available (0.5 arc minute resolution). We extracted the principal components of climate variation across our study sites using the prcomp function and the raster package (Hijmans and van Etten, 2012) in R (R Development Core Team, 2013). Parameter axes were scaled and centred prior to principal component analysis (PCA) and variable BIO14 (precipitation of driest month) was omitted since it was zero for all sites (i.e. invariant). Because the first two PCs from this analysis explained almost all (>94%) of the variation in climate among populations, we focus our analyses on these two axes.

However, we also consider in some cases overall climatic distances between pairs of populations, which were calculated with R's dist function as Euclidean distances using all principal components.

Univariate statistical analyses

We used bivariate Pearson correlation to test if climatic PC scores were correlated with mean population-level host preference, mean population-level mate preference, longitude or latitude. We used independent samples t-tests to determine whether climatic PC scores differed between populations using different host-plant species (*Ceanothus* vs. *Adenostoma*).

Multivariate statistical analyses

We conducted multivariate analyses testing the joint effects of host plant, geographic arrangement of populations (allopatry vs. sympatry), latitude, longitude, climate PC1, and climate PC2 on mean population-level host preference. We then did the same for mate preference. Due to the large number of independent variables considered, we used backward elimination to produce a reduced model, and concentrate on the results of this model. Due to *a priori* knowledge that even among parapatric populations the degree of reinforcement of mating preferences varies quantitatively with levels of gene flow, we also conducted analyses replacing the allopatry/sympatry term with the continuous variable of the proportion of an entire study site (size of the host plant patch of the focal population plus size of the host plant patch of the adjacent population on the alternative host) occupied by the population adjacent to the focal one. Host-plant patch size is positively correlated with *T. cristinae* population size and thus this index has been shown from both field and molecular studies to be a reliable index of gene flow, with gene flow into a population increasing as the patch size occupied by the adjacent population becomes larger in relative size (Sandoval, 1994b; Nosil *et al.*, 2003) (Fig. 2).

RESULTS

Climatic data

PC1 and PC2 explained 70% and 24% of the variance in climatic conditions among populations respectively. PC1 was interpretable as an index of temperature and precipitation variability (Table 3). For example, PC1 exhibited high negative loading for annual mean temperature and high positive loading for annual precipitation. Thus, high scores of PC1 are indicative of cold and wet climatic conditions and low PC1 scores of hot and dry conditions. Notably, the climatic PC scores did not differ between populations using different host species (PC1: $t_{10} = 1.21$, $P = 0.256$; PC2: $t_{10} = 0.46$, $P = 0.658$; t-tests) and were not correlated with either longitude ($r = 0.18$ and 0.46 for PC1 and PC2 respectively, both $P > 0.10$) or latitude ($r = 0.12$ and -0.21 for PC1 and PC2 respectively, both $P > 0.10$). Thus, in this dataset at least, host and geographic position are relatively independent of climate.

Univariate results

Both climate PCs were negatively correlated with mean preference for the host *Ceanothus* (PC1: $r = -0.80$, $P = 0.002$; PC2: $r = -0.71$, $P = 0.011$; both $n = 12$). In other words, mean preference for *Ceanothus* decreased as climates became wetter and colder. In strong contrast, neither PC was correlated with mean mate preference (PC1: $r = -0.00$, $P = 0.995$; PC2: $r = -0.03$, $P = 0.918$; both $n = 12$).

Multivariate results

For host preference, the reduced model derived using backward elimination retained three terms, one of which was climate PC1 (climate PC1: $B = -1.86$, s.e. = 0.50, $P = 0.006$; longitude: $B = -117.62$, s.e. = 55.18, $P = 0.066$; host: $B = -7.72$, s.e. = 3.23, $P = 0.056$). For mate preference, no terms were retained in the reduced model in the analysis where geography was coded as allopatry versus parapatry (all $P > 0.10$). Replacing the 'allopatry/parapatry' term with the quantitative measure of gene flow recovered the quadratic relationship between mate preference and gene flow reported previously (quadratic term: $B = -7.87$, s.e. = 0.333, $P = 0.042$), but no other factors were retained in the reduced model (all $P > 0.10$).

DISCUSSION

In this study, we examined the effects of host-plant use, climatic conditions, and the geographic arrangement of populations on reproductive isolation caused by divergent host-plant feeding and mating preferences. We found unique effects of each of these factors on reproductive isolation such that geographic variation in host-plant and mating preference represents a complex, but predictable, mosaic across the landscape. Such a pattern has also been documented for genomic divergence (Nosil *et al.*, 2012a, 2012b). We discuss in more detail below the effects of these selective factors, coupled with patterns of gene flow, on each type of preference and on genomic divergence and put our results in the context of related work in other study systems. We stress that our results are based on comparisons of population means, and thus further work examining the effects of climatic factors such as temperature on individual behavioural decisions is required.

Host-plant preferences

Past work on *T. cristinae* showed that individuals of both ecotypes often prefer to rest upon *Ceanothus* over *Adenostoma* in behavioural choice trials, but that this preference was stronger for the *Ceanothus* ecotype (Nosil *et al.*, 2006b; Nosil, 2007). The present results are consistent with this past work, via the marginally non-significant ($P = 0.056$) effect of host use on host preference in our reduced regression model. We also show here that climatic conditions affect host preference. Specifically, mean preference for *Ceanothus* increased among populations with increases in temperature and aridity (Fig. 3). This effect occurred for both ecotypes such that habitat isolation between ecotypes was not increased. A reasonable interpretation for this pattern is that selection for preference for the more nutritional host *Ceanothus* is stronger in harsher hot and dry climates, and that this occurs for both ecotypes. Further work testing this hypothesis is warranted, but two non-mutually exclusive processes could explain this pattern mechanistically. First, harsher climates could directly influence *T. cristinae* dietary requirements due to physiological changes in the insects. Second, harsher climates could result in morphological, physiological or phenological changes in *Ceanothus* (or *Adenostoma*) that make *Ceanothus* even more desirable for both ecotypes (for reviews, see Harrington *et al.*, 1999; Hughes, 2000; Cornelissen, 2011).

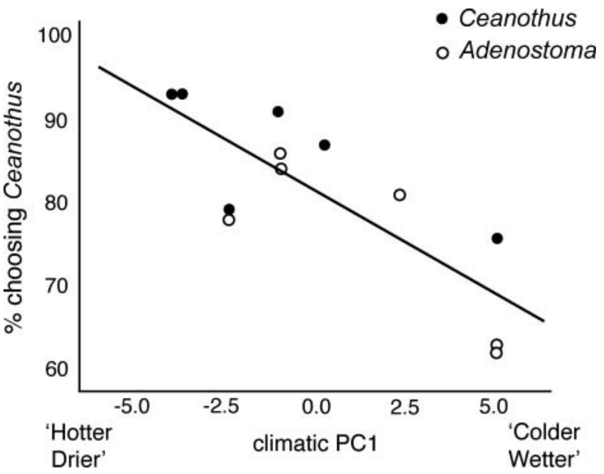

Fig. 3. Host preference varies among populations according to climatic conditions. The figure shows the relationship between mean host preference of a population (percentage of individuals within a population choosing *Ceanothus* over *Adenostoma* to rest upon in behavioural choice trials) and climatic conditions at the population locality. See text for details and statistics.

Another issue requiring further study is potential direct environmental effects of climate on host preference (i.e. phenotypic plasticity). Host preference differences among populations of *T. cristinae* are known to be at least partially heritable from reciprocal rearing and crossing studies. For example, F_1 hybrids between the ecotypes exhibit intermediate preferences between the parental types, and host preference differences among populations not only persist under common-garden conditions, but also appear unaffected by whether individuals are reared on *Ceanothus* or *Adenostoma* (Nosil *et al.*, 2006a, 2006b). However, these previous rearing experiments tested the effect of host-plant environment, not climate, on host preference. Thus, environmental effects of climate on host preference require further study, as do potential genotype × environment interactions (Rodríguez, 2013). Notably, environmentally induced host preferences are known to generate reproductive isolation in other herbivorous insect systems (Wood and Guttman, 1982; Wood and Keese, 1990; Drès and Mallet, 2002; Matsubayashi *et al.*, 2010).

How do our results on host preference fit with past work in other systems? In fact, they are opposite to those on host/food preferences in caterpillars (*Thaumetopoea wilkinsoni*), which become less host selective with increasing temperature (Ipekdal and Caglar, 2012), and also differ from results obtained for large pine weevils (*Hylobius abietis*) that show similarly strong host preferences at high and low temperatures, but relaxed host preferences at intermediate temperatures (Leather *et al.*, 1994). Other studies on changes in host/feeding preferences as a result of changing temperature were even more stochastic (Sotka and Giddens, 2009). This lends further support to the notion that climatic effects on host preferences in plant herbivores will be species- and context-specific (Bale *et al.*, 2002).

Mating preferences

In contrast to the effects of climate on host preference, across all 12 populations we did not detect effects of climate on mean mating preference. At first sight, this is somewhat surprising, because temperature and precipitation can dramatically affect the strength and direction of sexual selection within and between populations (Andersson and Simmons, 2006; Cornwallis and Uller, 2010; Robinson *et al.*, 2012). For example, female frogs have temperature-specific preferences for certain male call frequencies (Gerhardt and Mudry, 1980), and temperature modulates reproductive behaviour in pipefish (*Syngnathus abaster*), eastern mosquitofish (*Gambusia holbrooki*), and *Drosophila* spp. (Schnebel and Grossfield, 1984; Wilson, 2005; Silva *et al.*, 2007). In socially monogamous birds, unpredictability and variability of annual climatic cycles is strongly correlated with increasing infidelity and divorce rates (Botero and Rubenstein, 2012). Moreover, laboratory experiments have demonstrated that local adaptation to climatic variables can directly result in sexual isolation in *Drosophila melanogaster* (Kilias *et al.*, 1980) and *D. ananassae* (Yadav and Yadav, 2012), for example due to temperature-specific changes in cuticular hydrocarbons (Markow and Toolson, 1990).

Our results in *T. cristinae*, which contrast those cited above, are likely explained by reinforcement of sexual isolation between adjacent parapatric populations that are most climatically similar. Thus, the strongest predictor of mating preference remains the balance between reinforcing selection and gene flow. Notably, host plant adaptation plays a key role in reinforcement by generating the ecologically based selection against hybrids that drives reinforcement (Nosil *et al.*, 2003; Nosil, 2007). Another factor that could affect reinforcement, but which has not been examined in the *T. cristinae* system, is the amount of time that populations have been in contact and hybridizing (Gabor *et al.*, 2013).

In the *T. cristinae* system, it is possible that climate affects mating preferences among allopatric populations but this was not recovered in our dataset, which considered only four allopatric populations and exhibited patterns driven by processes acting in parapatry. This is a reasonable hypothesis given that past work showed mating preferences are divergent along different trait axes for allopatric versus parapatric populations (Nosil and Hohenlohe, 2012). Also consistent with this suggestion, pairwise estimates of reproductive isolation among the four allopatric populations considered here ($n = 6$ pairwise comparisons) increase with greater climatic distance between populations. However, this pattern is currently impossible to interpret because for these populations divergence in climate and host-plant use are strongly confounded (Table 4). Thus, studies of additional allopatric populations that vary independently in host-plant use and climate are required to test the extent to which divergence in host use, climate or both affects sexual isolation between allopatric populations.

Table 4. Pairwise estimates of sexual isolation (measured via the I_{PSI} index), climatic distance, and host-plant divergence among the four allopatric populations of *T. cristinae* considered here

Population pair	I_{PSI}	Climatic distance	Host use
P × LA	0.53	9.20	Different hosts
P × VPC	0.24	3.85	Same host
P × PRC	0.35	3.11	Same host
LA × VPC	0.43	6.74	Different hosts
LA × PRC	0.39	9.35	Different hosts
VPC × PRC	0.33	3.56	Same host

Note: Host-plant differences and climatic differences are confounded for this subset of populations (but not among all 12 populations, see Results).

Genomic divergence

Recent evidence implicates climate-related natural selection as a major driver of genomic divergence among populations of *T. cristinae*. In addition, host-plant use, reinforcement, and gene flow also each affect genomic divergence (Nosil *et al.*, 2012a, 2012b). We focus our discussion here on climatic effects, as these represent the new data and analyses in the current study. Climate has been implicated in genomic divergence in a number of other systems. In *Arabidopsis thaliana*, fitness-associated loci identified in field experiments show variation among populations according to climate (Fournier-Level *et al.*, 2011). Indeed, populations of *A. thaliana* from across Europe show genomic divergence correlated with climate differences between sites, and the loci most strongly correlated with climate were enriched in non-synonymous substitutions, suggesting they evolved by climate-driven natural selection (Hancock *et al.*, 2011). Non-synonymous substitutions also constituted a considerable proportion of climate-correlated SNPs found between black spruce (*Picea mariana*) populations (Prunier *et al.*, 2011). In the ocean, temperature clines appear to drive the parallel divergence of multiple independent genes in cod (*Gadus morhua*) in the eastern and western North Atlantic (Bradbury *et al.*, 2010), and climate also explains a greater proportion of genomic divergence than host-plant use in the large pine weevil (*Hylobius abietis*) (Manel *et al.*, 2009). Although some of these studies used only a moderate number of markers by today's standards, genomic divergence due to adaptation to climate appears to be rather widespread across the genome and may be a general phenomenon.

Conclusions and future work

In conclusion, multiple selective processes and gene flow combine to affect patterns of geographic variation in behaviour in *T. cristinae*. In Table 5, we summarize the relative effects of these different processes on host preference, mate preference, and genomic divergence. Notably, *T. cristinae* is only one of more than 20 species in the genus *Timema* (Crespi and Sandoval, 2000). Thus, further studies in other species, which are known to inhabit a wider range of both host-plant use and climatic conditions than populations of *T. cristinae*, are now required to test the extent to which the patterns observed across the genus mirror those seen in *T. cristinae*. If they largely do so, but in an exaggerated manner, it would indicate that the adaptive radiation of *Timema* represents the micro-evolutionary processes and patterns seen in *T. cristinae* 'writ large'.

Table 5. Summary of the effect of multiple sources of selection and gene flow between populations on different hosts on behavioural reproductive isolation caused by divergent mating and host preferences and patterns of genomic divergence

	Host adaptation	Climate	Reinforcement	Gene flow
Host preference	Moderate	***Strong***	Weak	Moderate
Mate preference	Moderate	Weak	***Strong***	***Strong***
Genome divergence	Moderate	***Strong***	***Strong***	***Strong***

Note: Categorization was delimited as follows: Strong = often overwhelms other factors; Moderate = is detectable but weaker than other effects; Weak = process occurring but not readily detectable, or not occurring

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REFERENCES

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N. *et al.* 2013. Hybridization and speciation. *J. Evol. Biol.*, **26**: 229–246.

Andersson, M. and Simmons, L.W. 2006. Sexual selection and mate choice. *Trends Ecol. Evol.*, **21**: 296–302.

Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K. *et al.* 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biol.*, **8**: 1–16.

Barton, N.H. 2001. The role of hybridization in evolution. *Mol. Ecol.*, **10**: 551–568.

Bolnick, D.I. and Doebeli, M. 2003. Sexual dimorphism and adaptive speciation: two sides of the same ecological coin. *Evolution*, **57**: 2433–2449.

Bolnick, D.I. and Fitzpatrick, B.M. 2007. Sympatric speciation: models and empirical evidence. *Annu. Rev. Ecol. Evol. Syst.*, **38**: 459–487.

Bolnick, D.I. and Nosil, P. 2007. Natural selection in populations subject to a migration load. *Evolution*, **61**: 2229–2243.

Bolnick, D.I., Caldera, E.J. and Matthews, B. 2008. Evidence for asymmetric migration load in a pair of ecologically divergent stickleback populations. *Biol. J. Linn. Soc.*, **94**: 273–287.

Botero, C.A. and Rubenstein, D.R. 2012. Fluctuating environments, sexual selection and the evolution of flexible mate choice in birds. *PLoS One*, **7**: e32311.

Bradbury, I.R., Hubert, S., Higgins, B., Borza, T., Bowman, S., Paterson, I.G. *et al.* 2010. Parallel adaptive evolution of Atlantic cod on both sides of the Atlantic Ocean in response to temperature. *Proc. R. Soc. Lond. B*, **277**: 3725–3734.

Cain, M.L., Andreasen, V. and Howard, D.J. 1999. Reinforcing selection is effective under a relatively broad set of conditions in a mosaic hybrid zone. *Evolution*, **53**: 1343–1353.

Carvajal-Rodriguez, A. and Rolan-Alvarez, E. 2006. JMATING: a software for the analysis of sexual selection and sexual isolation effects from mating frequency data. *BMC Evol. Biol.*, **6**: 40.

Cockburn, A., Osmond, H.L. and Double, M.C. 2008. Swingin' in the rain: condition dependence and sexual selection in a capricious world. *Proc. R. Soc. Lond. B*, **275**: 605–612.

Comeault, A.A., Soria-Carrasco, V., Gompert, Z., Farkas, T.E., Buerkle, C.A., Parchman, T.L. *et al.* submitted. Genome-wide association mapping of phenotypic traits subject to a range of intensities of natural selection in *Timema cristinae*.

Cornelissen, T. 2011. Climate change and its effects on terrestrial insects and herbivory patterns. *Neotrop. Entomol.*, **40**: 155–163.

Cornwallis, C.K. and Uller, T. 2010. Towards an evolutionary ecology of sexual traits. *Trends Ecol. Evol.*, **25**: 145–152.

Coyne, J.A. and Orr, H.A. 2004. *Speciation*. Sunderland, MA: Sinauer Associates.

Crespi, B.J. 2000. The evolution of maladaptation. *Heredity*, **84**: 623–629.

Crespi, B.J. and Sandoval, C.P. 2000. Phylogenetic evidence for the evolution of ecological specialization in *Timema* walking-sticks. *J. Evol. Biol.*, **13**: 249–262.

Drès, M. and Mallet, J. 2002. Host races in plant-feeding insects and their importance in sympatric speciation. *Phil. Trans. R. Soc. Lond. B*, **357**: 471–492.

Endler, J.A. 1973. Gene flow and population differentiation. *Science*, **179**: 243–250.

Endler, J.A. 1977. *Geographic Variation, Speciation, and Clines*. Princeton, NJ: Princeton University Press.

Fournier-Level, A., Korte, A., Cooper, M.D., Nordborg, M., Schmitt, J. and Wilczek, A.M. 2011. A map of local adaptation in *Arabidopsis thaliana*. *Science*, **333**: 86–89.

Gabor, C.R., Alberici da Barbiano, L. and Aspbury, A.S. 2013. Geographic variation in male mate choice in a gynogenetic species complex: evaluating long-term data across mating contexts. *Evol. Ecol. Res.*, **15**: 653–666.

Gerhardt, H.C. 2013. Geographic variation in acoustic communication: reproductive character displacement and speciation. *Evol. Ecol. Res.*, **15**: 605–632.

Gerhardt, H.C. and Mudry, K.M. 1980. Temperature effects on frequency preferences and mating call frequencies in the green treefrog, *Hyla cinerea* (Anura, Hylidae). *J. Comp. Physiol.*, **137**: 1–6.

Hancock, A.M., Brachi, B., Faure, N., Horton, M.W., Jarymowycz, L.B., Sperone, F.G. *et al.* 2011. Adaptation to climate across the *Arabidopsis thaliana* genome. *Science*, **333**: 83–86.

Harrington, R., Woiwod, I. and Sparks, T. 1999. Climate change and trophic interactions. *Trends Ecol. Evol.*, **14**: 146–150.

Hendry, A.P. and Taylor, E.B. 2004. How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake–stream stickleback pairs. *Evolution*, **58**: 2319–2331.

Hendry, A.P., Day, T. and Taylor, E.B. 2001. Population mixing and the adaptive divergence of quantitative traits in discrete populations: a theoretical framework for empirical tests. *Evolution*, **55**: 459–466.

Hendry, A.P., Bolnick, D.I., Berner, D. and Peichel, C.L. 2009. Along the speciation continuum in sticklebacks. *J. Fish Biol.*, **75**: 2000–2036.

Hijmans, R.J. and van Etten, J. 2012. *raster: Geographic Data Analysis and Modeling*. R package version 2.0-41. http://CRAN.R-project.org/package = raster.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. and Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.*, **25**: 1965–1978.

Hughes, L. 2000. Biological consequences of global warming: is the signal already apparent? *Trends Ecol. Evol.*, **15**: 56–61.

Ipekdal, K. and Caglar, S.S. 2012. Effects of temperature on the host preference of pine processionary caterpillar *Thaumetopoea wilkinsoni* Tams, 1924 (Lepidoptera: Notodontidae). *Turk. J. Zool.*, **36**: 319–328.

Kilias, G., Alahiotis, S.N. and Pelecanos, M. 1980. A multifactorial genetic investigation of speciation theory using *Drosophila melanogaster. Evolution*, **34**: 730–737.

Kirkpatrick, M. 2000. Reinforcement and divergence under assortative mating. *Proc. R. Soc. Lond. B*, **267**: 1649–1655.

Langerhans, R.B. and Makowicz, A.M. 2013. Sexual selection paves the road to sexual isolation during ecological speciation. *Evol. Ecol. Res.*, **15**: 633–651.

Leather, S.R., Ahmed, S.I. and Hogan, L. 1994. Adult feeding preferences of the large pine weevil, *Hylobius abietis* (Coleoptera, Curculionidae). *Eur. J. Entomol.*, **91**: 385–389.

Lowry, D.B., Modliszewski, J.L., Wright, K.M., Wu, C.A. and Willis, J.H. 2008. The strength and genetic basis of reproductive isolating barriers in flowering plants. *Phil. Trans. R. Soc. Lond. B*, **363**: 3009–3021.

Manel, S., Conord, C. and Despres, L. 2009. Genome scan to assess the respective role of host-plant and environmental constraints on the adaptation of a widespread insect. *BMC Evol. Biol.*, **9**: 288.

Markow, T.A. and Toolson, E.C. 1990. Temperature effects on epicuticular hydrocarbons and sexual isolation in *Drosophila mojavensis*. In *Ecological and Evolutionary Genetics of Drosophila* (J.F.S. Barker, W.T. Starmer and R.J. MacIntyre, eds.), pp. 315–331. New York: Plenum Press.

Matsubayashi, K.W. and Katakura, H. 2009. Contribution of multiple isolating barriers to reproductive isolation between a pair of phytophagous ladybird beetles. *Evolution*, **63**: 2563–2580.

Matsubayashi, K.W., Ohshima, I. and Nosil, P. 2010. Ecological speciation in phytophagous insects. *Entomol. Exp. Appl.*, **134**: 1–27.

Nosil, P. 2004. Reproductive isolation caused by visual predation on migrants between divergent environments. *Proc. R. Soc. Lond. B*, **271**: 1521–1528.

Nosil, P. 2007. Divergent host plant adaptation and reproductive isolation between ecotypes of *Timema cristinae* walking sticks. *Am. Nat.*, **169**: 151–162.

Nosil, P. 2008. Ernst Mayr and the integration of geographic and ecological factors in speciation. *Biol. J. Linn. Soc.*, **95**: 26–46.

Nosil, P. 2009. Adaptive population divergence in cryptic color-pattern following a reduction in gene flow. *Evolution*, **63**: 1902–1912.

Nosil, P. 2012. *Ecological Speciation*. Oxford: Oxford University Press.

Nosil, P. and Crespi, B.J. 2004. Does gene flow constrain adaptive divergence or vice versa? A test using ecomorphology and sexual isolation in *Timema cristinae* walking-sticks. *Evolution*, **58**: 102–112.

Nosil, P. and Crespi, B.J. 2006. Experimental evidence that predation promotes divergence in adaptive radiation. *Proc. Natl. Acad. Sci. USA*, **103**: 9090–9095.

Nosil, P. and Hohenlohe, P.A. 2012. Dimensionality of sexual isolation during reinforcement and ecological speciation in *Timema cristinae* stick insects. *Evol. Ecol. Res.*, **14**: 467–485.

Nosil, P. and Yukilevich, R. 2008. Mechanisms of reinforcement in natural and simulated polymorphic populations. *Biol. J. Linn. Soc.*, **95**: 305–319.

Nosil, P., Crespi, B.J. and Sandoval, C.P. 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature*, **417**: 440–443.

Nosil, P., Crespi, B.J. and Sandoval, C.P. 2003. Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. *Proc. R. Soc. Lond. B*, **270**: 1911–1918.

Nosil, P., Vines, T.H. and Funk, D.J. 2005. Perspective: Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution*, **59**: 705–719.

Nosil, P., Crespi, B.J., Sandoval, C.P. and Kirkpatrick, M. 2006a. Migration and the genetic covariance between habitat preference and performance. *Am. Nat.*, **167**: E66–E78.

Nosil, P., Sandoval, C.P. and Crespi, B.J. 2006b. The evolution of host preference in allopatric vs. parapatric populations of *Timema cristinae* walking-sticks. *J. Evol. Biol.*, **19**: 929–942.

Nosil, P., Egan, S.P. and Funk, D.J. 2008. Heterogeneous genomic differentiation between walking-stick ecotypes: 'isolation by adaptation' and multiple roles for divergent selection. *Evolution*, **62**: 316–336.

Nosil, P., Harmon, L.J. and Seehausen, O. 2009. Ecological explanations for (incomplete) speciation. *Trends Ecol. Evol.*, **24**: 145–156.

Nosil, P., Gompert, Z., Farkas, T.E., Comeault, A.A., Feder, J.L., Buerkle, C.A. *et al.* 2012a. Genomic consequences of multiple speciation processes in a stick insect. *Proc. R. Soc. Lond. B*, **279**: 5058–5065.

Nosil, P., Parchman, T.L., Feder, J.L. and Gompert, Z. 2012b. Do highly divergent loci reside in genomic regions affecting reproductive isolation? A test using next-generation sequence data in *Timema* stick insects. *BMC Evol. Biol.*, **12**: 164.

Pröhl, H., Willink, B. and Hauswaldt, S. 2013. Geographic variation in sexual signals and behaviour in two species of poison frogs. *Evol. Ecol. Res.*, **15**: 667–687.

Prunier, J., Laroche, J., Beaulieu, J. and Bousquet, J. 2011. Scanning the genome for gene SNPs related to climate adaptation and estimating selection at the molecular level in boreal black spruce. *Mol. Ecol.*, **20**: 1702–1716.

R Development Core Team. 2013. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Robinson, M.R., van Doorn, G.S., Gustafsson, L. and Qvarnstrom, A. 2012. Environment-dependent selection on mate choice in a natural population of birds. *Ecol. Lett.*, **15**: 611–618.

Rodríguez, R.L. 2013. Causes of variation in genotype × environment interaction. *Evol. Ecol. Res.*, **15**: 733–746.

Safran, R.J., Vitousek, M.N., Hauber, M.E. and Ghalambor, C.K. 2010. Sexual selection: a dynamic state of affairs. Response to the comments of Cornwallis and Uller in the article: Towards an evolutionary ecology of sexual traits. *Trends Ecol. Evol.*, **25**: 429–430.

Sanderson, N. 1989. Can gene flow prevent reinforcement. *Evolution*, **43**: 1223–1235.

Sandoval, C.P. 1993. Geographic, ecological and behavioral factors affecting spatial variation in color or morph frequency in the walking-stick *Timema cristinae*. PhD thesis, University of California.

Sandoval, C.P. 1994a. Differential visual predation on morphs of *Timema cristinae* (Phasmatodeae, Timemidae) and its consequences for host-range. *Biol. J. Linn. Soc.*, **52**: 341–356.

Sandoval, C.P. 1994b. The effects of relative geographical scales of gene flow and selection on morph frequencies in the walking-stick *Timema cristinae. Evolution*, **48**: 1866–1879.

Sandoval, C.P. and Nosil, P. 2005. Counteracting selective regimes and host preference evolution in ecotypes of two species of walking-sticks. *Evolution*, **59**: 2405–2413.

Schluter, D. 1988. Estimating the form of natural selection on a quantitative trait. *Evolution*, **42**: 849–861.

Schluter, D. 2009. Evidence for ecological speciation and its alternative. *Science*, **323**: 737–741.

Schnebel, E.M. and Grossfield, J. 1984. Mating-temperature range in *Drosophila. Evolution*, **38**: 1296–1307.

Servedio, M.R. and Kirkpatrick, M. 1997. The effects of gene flow on reinforcement. *Evolution*, **51**: 1764–1772.

Servedio, M.R. and Noor, M.A.F. 2003. The role of reinforcement in speciation: theory and data. *Annu. Rev. Ecol. Evol. Syst.*, **34**: 339–364.

Silva, K., Vieira, M.N., Almada, V.C. and Monteiro, N.M. 2007. The effect of temperature on mate preferences and female–female interactions in *Syngnathus abaster. Anim. Behav.*, **74**: 1525–1533.

Sotka, E.E. and Giddens, H. 2009. Seawater temperature alters feeding discrimination by cold-temperate but not subtropical individuals of an ectothermic herbivore. *Biol. Bull.*, **216**: 75–84.

Thompson, J.N. 2005. *The Geographic Mosaic of Coevolution*. Chicago, IL: University of Chicago Press.

Wilson, R.S. 2005. Temperature influences the coercive mating and swimming performance of male eastern mosquitofish. *Anim. Behav.*, **70**: 1387–1394.

Wood, T.K. and Guttman, S.I. 1982. Ecological and behavioral basis for reproductive isolation in the sympatric *Enchenopa binotata* complex (Homoptera, Membracidae). *Evolution*, **36**: 233–242.

Wood, T.K. and Keese, M.C. 1990. Host-plant induced assortative mating in *Enchenopa* treehoppers. *Evolution*, **44**: 619–628.

Yadav, S. and Yadav, A.K. 2012. Effect of temperature on behavioural isolation: a study with *Drosophila ananassae* populations. *Indian J. Exp. Biol.*, **50**: 366–371.