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The role of selection and gene flow in the evolution of sexual isolation in *Timema* walking sticks and other Orthopteroids

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Abstract

The formation of new species involves the evolution of barriers to gene exchange. One such barrier is sexual isolation, where divergent mate preferences prevent copulation between taxa. Sexual isolation can evolve via a number of processes, including natural selection, sexual selection, genetic drift, and reinforcing selection to avoid maladaptive hybridization. Conversely, gene flow between populations generally erodes the evolution of sexual isolation. In *Timema cristinae* walking stick insects, some sexual isolation between populations evolved through ecological divergence in host-plant use, implicating a role for natural selection. However, reinforcement and gene flow also contribute, such that sexual isolation is strongest when migration between hosts is high enough to result in selection to avoid hybridization, but low enough to prevent gene flow from eroding adaptive divergence in mate choice. Both parallel and contrasting patterns can be seen in other Orthopteroids. This variation among groups might reflect 1) the degree to which different groups are intimately associated with their food resources, 2) the types of traits used in mate choice, and 3) how the geographic arrangement of populations affects the opportunity for reinforcement.

Key words

speciation, reproductive isolation, reinforcement, hybridization, gene flow, Orthoptera, Phasmidae

Introduction

Speciation involves the evolution of reproductive isolation between diverging populations. Understanding speciation thus requires determining which reproductive barriers initially reduced gene flow between populations and the evolutionary forces producing them (Mayr 1947, 1963; Coyne & Orr 2004). The evolution of premating isolation caused by divergent mating signals and preferences (sexual isolation hereafter) appears to be an important component of speciation in many taxa (Coyne & Orr 2004). However, questions remain about which processes tend to promote, versus constrain, the evolution of sexual isolation. Here I review the role of 3 processes in the evolution of sexual isolation between populations of *Timema* walking stick insects: ecological adaptation, reinforcement, and gene flow. I conclude by comparing the results from walking sticks to those from studies of other orthopteroids.

Speciation occurs as an indirect by-product of ecological divergence when barriers to gene flow evolve between populations as a result of ecologically based divergent selection (Mayr 1947, 1963; Schluter 2000; Jiggins *et al.* 2001). Natural selection causes population divergence in ecologically important traits and these traits (or traits correlated with them) also incidentally cause reproductive isolation. When speciation occurs as a by-product of

adaptive divergence, ecologically divergent populations exhibit greater reproductive isolation than ecologically similar populations of similar age ('ecological speciation'—Funk 1998, Rundle *et al.* 2000, Schluter 2000, Funk *et al.* 2002, Rundle & Nosil 2005). The evolution of sexual isolation due to habitat-specific divergent selection on preferences themselves (rather than ecological traits *per se*) generates the same prediction, and also involves ecological divergence (Endler 1992, Boughman 2002).

Speciation can also involve selection for sexual isolation in geographical regions where hybridization is maladaptive (*i.e.*, reinforcement) (Dobzhansky 1937; see Butlin 1995, Howard 1993, Noor 1999, Servedio & Noor 2003 for reviews). The key prediction of the reinforcement hypothesis is that nonallopatric (geographically contiguous or overlapping) populations will exhibit greater mating discrimination than allopatric (geographically separated) populations, because selection against hybridization can only occur in the former. This pattern of greater divergence in sympatry/parapatry is often called 'reproductive character displacement', and previous empirical studies of reinforcement have provided evidence for such a pattern (Noor 1995, Saetre *et al.* 1997, Rundle & Schluter 1998, Higgin *et al.* 2000). However, it is unclear how ecological adaptation and reinforcement interact during the speciation process (Schluter 2000, Rundle & Nosil 2005).

Gene flow between populations tends to erode divergence and thus prevent speciation (Mayr 1947, Slatkin 1987, Sanderson 1989, Servedio & Kirkpatrick 1997, Cain *et al.* 1999). Thus high hetero-specific encounter rates might be expected to prevent the evolution of reproductive barriers. However, heterospecific encounters also provide the opportunity for the selection against hybridization that reinforcement requires (Howard 1993, Noor 1995). Thus the likelihood of reinforcement might be greatest when population sizes are similar and migration rates are intermediate (Sanderson 1989, Servedio & Kirkpatrick 1997, Kirkpatrick 2000).

There have been studies of the evolution of sexual isolation between populations of walking sticks, populations which differ in ecology (host plant use), geography (allopatry *vs* parapatry) and population size. This variation allows partitioning of the effects of ecology and geography on reproductive isolation and provides the opportunity to explicitly test the predictions outlined above. The results show that the greatest levels of sexual isolation occur under the combined effects of ecological adaptation and reinforcement, whereas high levels of gene flow erode divergence.

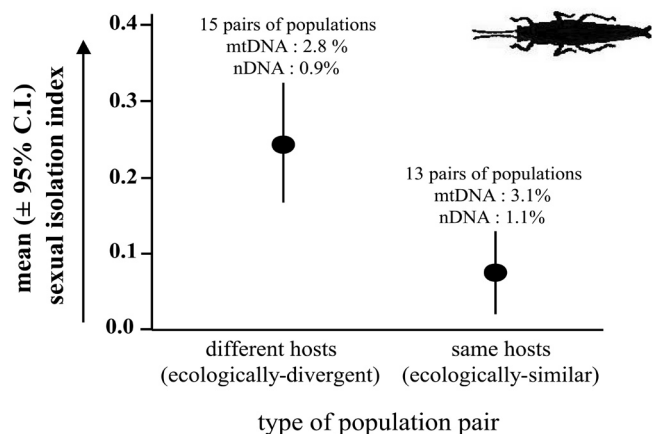


Fig. 1. The degree of sexual isolation is greater between ecologically divergent pairs of walking stick populations, than between ecologically similar pairs of populations of similar age ($p < 0.05$, Mantel test). Shown is mean sexual isolation averaged across population pairs within each comparison, with 95% confidence intervals (C.I.). Conversely, mean genetic distance did not differ between populations using the same, versus different, hosts (mtDNA and nDNA values refer to the average genetic distance between population pairs in mitochondrial DNA and in an independently evolving nuclear locus respectively; Mantel test $t = 0.47, 0.76$, respectively, both $p > 0.15$). Sexual isolation is measured using an index where zero indicates random mating and 1 indicates complete sexual isolation. Data are from Nosil *et al.* (2002).

Study System

Timema walking sticks are wingless, phytophagous insects inhabiting the chaparral of southwestern North America (Crespi & Sandoval 2000). Individuals feed and mate exclusively on the hosts upon which they rest. Most work has focused on *T. cristinae*, a species feeding upon 2 different host plant species (*Ceanothus spinosus* and *Adenostoma fasciculatum*). A 'population' of walking sticks is defined as all the individuals collected within a homogenous patch of a single host species (as in Nosil *et al.* 2002, 2003; Nosil 2004). Thus 'parapatric' insect populations are in contact with a population of insects adapted to the alternative host (*i.e.*, they have an adjacent population using the alternative host), whereas 'allopatric' populations are geographically separated from all other populations adapted to the alternative host by distances $> 50 \times$ the 12-m per-generation gene-flow distance (Sandoval 1993). Sample sites with both hosts were chosen such that there was only one population on each host species (*i.e.*, each population had only one adjacent population on the alternate host). For simplicity, I use the term 'hybridization' to refer to interbreeding between populations on different hosts, but do not imply that the host forms have achieved full species status.

Ecological divergence and the evolution of sexual isolation

Pairs of populations using the same host species can be considered 'ecologically similar', whereas those using different host species can be considered 'ecologically divergent'. Whether sexual isolation was greater between pairs of populations that use the same, *vs* different, host plant species, was tested by Nosil *et al.* (2002). A total of 1024 no-choice mating trials were conducted between 28 pairs of populations, with 13 pairs using the same host species

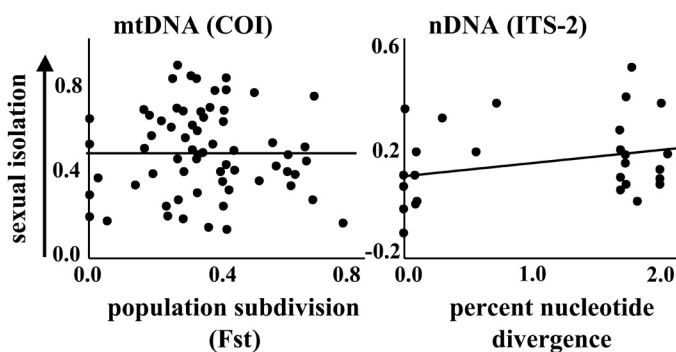


Fig. 2. The magnitude of sexual isolation observed between pairs of walking stick populations is independent of the genetic distance between pairs of populations (mtDNA: $r = 0.13$, $p = 0.29$; nDNA: $r = 0.29$, $p = 0.10$; Mantel tests). Sexual isolation is measured using an index where zero indicates random mating and 1 indicates complete sexual isolation. Genetic distance is calculated from DNA sequence variation at 2 independently evolving loci (mtDNA—mitochondrial locus COI; nDNA—nuclear locus ITS-2). The mtDNA data are from the 66 pairs of populations examined in Nosil *et al.* (2003) and the nDNA data from the 28 pairs examined in Nosil *et al.* (2002). The lines are least-squares regression lines, whereas significance testing was conducted using Mantel tests due to nonindependence among data points.

and 15 pairs using different host plant species. Consistent with the ecological speciation hypothesis, walking sticks were more likely to copulate if paired with an opposite-sex member from the same host-plant species, than if paired with an opposite-sex member from a different host plant species. Thus the magnitude of sexual isolation detected between pairs of populations using different host plants was significantly greater than the magnitude of isolation detected between pairs of populations using the same host plant (Fig. 1; mean I_{PSI} isolation index = 0.24 *vs* 0.08 respectively; Mantel test $t = 2.24$; $p < 0.05$). Notably, a reciprocal rearing experiment showed that sexual isolation between populations is independent from the host plant species that individuals are reared upon, indicating that sexual isolation likely has a strong genetic basis (Nosil *et al.* 2003).

Time since divergence is unlikely to confound the results reported above because the degree of sexual isolation observed between populations was not correlated with the genetic distance between pairs of populations (Fig. 2). Moreover, pairs of populations using different host plants were not more genetically divergent from one another than pairs of populations using the same host plant (Fig. 1). Thus ecological divergence in host plant use, rather than neutral differentiation, predicts the magnitude of sexual isolation that evolves.

Reinforcement of sexual isolation

The main prediction of reinforcement was supported; female mating discrimination against foreign males (*i.e.*, males from other populations) is significantly stronger when females are from populations where the 2 host-adapted forms are in geographic contact (*i.e.*, parapatry) than when females are from geographically isolated populations (*i.e.*, allopatry) (Fig. 3; mean between-population copulation frequencies in mating trials involving females from parapatric populations = 28%, $s_{\bar{x}} = 0.45$; in trials using females from allopatric populations = 35%, $s_{\bar{x}} = 0.47$; $p < 0.001$ in a logistic regression analysis) (Nosil *et al.* 2003).

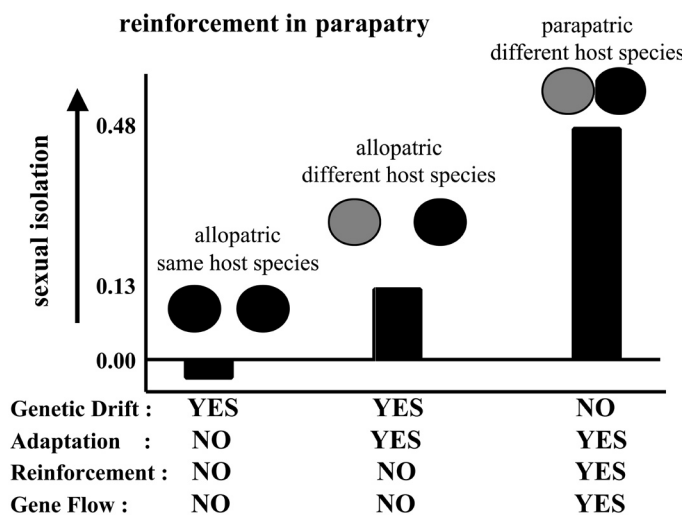


Fig. 3. Evidence for reproductive character displacement in *T. cristinae* walking sticks. The degree of female mating discrimination against males from other populations (*i.e.*, 'foreign males') is greatest when populations adapted to a different host co-occur geographically in parapatry (y-axis shows the value of mean copulation frequency with males from the females own population, minus the mean copulation frequency with foreign males, where data from all the between-population mating trials within an ecogeographic comparison are pooled). Depicted below the x-axis are the evolutionary forces likely to be acting in each of the 3 ecogeographic comparisons, (see text for details). Data are from Nosil *et al.* (2002, 2003).

To be relatively certain that reinforcement is the process which caused the pattern of reproductive character displacement documented above, three requirements must be met. First, a history of hybridization is required. Both morphological and molecular data indicate that gene flow (*i.e.*, hybridization) does occur between adjacent pairs of populations on different hosts. Adjacent pairs of populations using different host plants are weakly or not differentiated at mtDNA (mean $F_{st} = 0.07$, range = 0.00 to 0.25, $n = 7$ pairs), while geographically-separated populations are more strongly differentiated (mean $F_{st} = 0.31$, range = 0.00 to 0.79, $n = 129$ pairs; Mantel's $t = 2.33$, $p < 0.01$). This result is not restricted to mtDNA, because the magnitude of divergence in color pattern, body size and shape, and host preference is also weaker between parapatric versus allopatic populations (Sandoval 1994a,b; Nosil & Crespi 2004; Nosil *et al.* 2006). These data suggest that gene flow between neighboring populations occurs in the wild (Coyne & Orr 2004).

Second, reinforcement traditionally requires reduced hybrid fitness. This precondition is likely fulfilled in *T. cristinae* because hybrid broods contain a higher proportion of color-pattern morphs that are locally noncryptic, than do broods derived from within-population mating (Sandoval 1993). Field experiments and predation trials have shown that natural selection against noncryptic morphs occurs, and is caused by differential visual predation (Sandoval 1994a,b; Nosil *et al.* 2003; Nosil 2004). Thus hybrids likely suffer higher rates of visual predation than do the parental forms, imposing selection to avoid hybridization. Additionally, females from some parapatric populations exhibit reduced fecundity and oviposition rate following between-population mating, relative to within-population mating (Nosil & Crespi 2006). This reduction in female fitness represents another cost to hybridization, and could also select for mating discrimination against males from the other host.

Third, alternative explanations for the pattern of reproductive character displacement need to be unlikely. Numerous processes other than reinforcement could cause a pattern of reproductive character displacement (Howard 1993, Butlin 1995, Noor 1999 for review). Each of these hypotheses can be viewed as an alternative to reinforcement and examples include ecological character displacement, population ancestry, biased extinction, and male preference for allopatic females. Each of these alternatives was examined, but was unsupported (Nosil *et al.* 2003 for details). Thus it is likely that reinforcement contributes to the observed pattern of reproductive character displacement.

Relative population size and reinforcement

The 8 parapatric populations studied by Nosil *et al.* (2003) exhibited a range of variation in the effects of reinforcement (*i.e.*, in the magnitude of sexual isolation). Relative population sizes can affect the likelihood and strength of reinforcement, and may explain some of this variation. Numerous theoretical models have demonstrated that high levels of gene flow between diverging populations can erode the effects of reinforcing selection, and thus prevent reinforcement (Sanderson 1989, Servedio & Kirkpatrick 1997, Cain *et al.* 1999, Servedio & Noor 2003 for review). However, although migration between divergent populations can result in gene flow between them, it also generates the opportunity for selection against hybridization to occur. Thus migration can exert a dual effect during reinforcement. On the one hand, increasing rarity of a population (*i.e.*, smaller size relative to the population with which it co-occurs) elevates the migration rate into the population. This process raises the probability of encountering mates from the other populations, and thus the opportunity for reinforcing selection (Howard 1993, Noor 1995). On the other hand, such increased opportunity for between-population mating also increases the potential for gene flow between populations, which retards reinforcement. The actual magnitude of mating discrimination that evolves might be expected to reflect a balance between the opposing forces of reinforcing selection and gene flow, with the effects of reinforcement being greatest when population sizes are similar and when migration rates are intermediate.

The dual effects of migration were given explicit theoretical consideration by Kirkpatrick (2000), who examined population divergence in mating preferences between a continent and an island receiving continental migrants. The per generation change in the mean value of a trait used as a cue for assortative mating in the island population (T), is given by the equation:

$$\text{Change in } T = GB + m(T' - T) - m(T' - T)I$$

where G is the additive genetic variance, B is the directional selection gradient acting on the assortment trait, m is the rate at which the island is receiving migrants and I is the intensity of selection against migrants and hybrids.

Two migration terms are represented: $m(T' - T)$ represents the homogenizing effects of gene flow, causing mean trait values between populations to become similar; $m(T' - T)I$ demonstrates that migration can also cause population divergence in mean trait values, by providing the opportunity for selection against migrants and hybrids, which acts as the force driving divergence.

Few empirical studies have examined the effects of migration on the outcome of reinforcement (see Servedio & Noor 2003 for review). The walking stick study of Nosil *et al.* (2003) explicitly tests

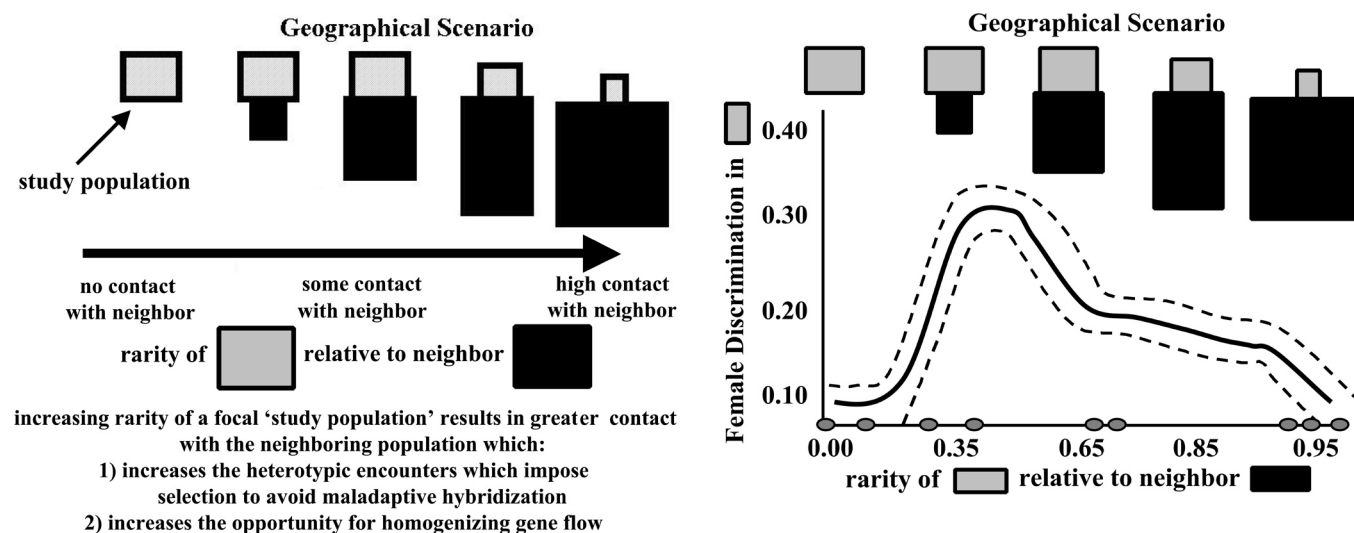


Fig. 4. Female mating discrimination against males from other populations is strongest when the rarity (*i.e.*, relative size) of the study population is intermediate (8 parapatric populations, 4 allopatric populations). Shown here is the relationship between the rarity of a study population (relative to its neighboring population of the alternative host; values for each study population denoted by black circles on the x-axis) and female mating discrimination against foreign males that use the alternative host (absolute value of mean copulation frequency with foreign males, minus mean copulation frequency with resident males, for each of the 12 study populations). All 4 allopatric populations have a value of zero on the x-axis, such that only 9 circles are depicted along that axis. Boxes illustrate the different geographical scenarios, where the grey box denotes the study population and the black box denotes the neighboring population. The curve was estimated using the nonparametric cubic spline (dashed lines show standard errors from 1000 bootstrap replicates) (Schluter 1988). The figures are modified from Nosil *et al.* (2003).

for and demonstrates, the dual effects of migration: the magnitude of female mating discrimination against males from other populations is greatest when migration rates between populations adapted to alternate host plants are high enough to allow the evolution of reinforcement, but low enough to prevent gene flow from eroding adaptive divergence in mate choice (*i.e.*, when the sizes of coexisting populations are similar; Fig 4). More specifically, female mating discrimination against males from the alternative host plant was quantified in multiple populations which differed in the relative size of a neighboring, adjacent population. The relative size of the adjacent population is estimated from host-plant patch size, and is positively correlated with mtDNA estimates of gene flow (Nosil *et al.* 2003). Consistent with the balancing effects of reinforcing selection and gene flow, mating discrimination is low when the study population is allopatric or large relative to its neighbor; it increases rapidly until the sizes of the study and neighboring populations are similar, and it then decreases when the study population is relatively rare (Fig. 4). The generality of the walking stick study is unknown, although at least 3 other studies have documented stronger effects of reinforcement when a species is relatively rarer (*i.e.*, relatively less abundant: Waage 1979, Noor 1995, Peterson *et al.* 2005). Further studies are required to generate insight into whether migration will tend to facilitate divergence via increased opportunity for reinforcing selection or to act as a homogenizing force.

Outstanding Questions

Collectively, the findings from *T. cristinae* indicate that both selection and gene flow affect the magnitude of sexual isolation observed in nature. The greatest levels of sexual isolation are observed under the combined effects of ecological adaptation and reinforcement (Fig. 3). There are few other empirical or theoretical studies of how ecology and reinforcement interact during speciation (but

see Rundle *et al.* 2000, Jiggins *et al.* 2001 for data and Kirkpatrick 2001 for theory).

A number of outstanding questions remain. For the host ecotypes of *T. cristinae*, the foremost question is which traits have diverged between populations to cause sexual isolation? For example, between-population mating probability is independent of differences between the sexes in color pattern, body size, and body shape (Nosil *et al.* 2002, Nosil & Crespi 2004). Preliminary data from gas chromatography and behavioral experiments, suggest that host-specific divergence in pheromones is involved (Nosil, Gries & Gries, unpub.), and further studies of olfactory communication are underway. Additionally, the potential role of courtship behavior is yet to be examined. Successful copulation in *T. cristinae* involves at least 2 distinct stages. First, the male must approach the female and attempt to mount her. Second, the female must allow the male to mount and copulate with her, as males cannot force copulation. A number of behaviors, such as antennal touching, kicking, and leg tapping by the males, are performed at both stages.

Other questions involve the forms of hybrid dysfunction that were most important for reinforcement. Theory predicts that the importance of different types of hybrid dysfunction depends on both the magnitude of hybrid dysfunction and on the genetic covariance between traits reducing hybrid fitness and mating preferences (Barton & Turelli 1991, Servedio 2001). Finally, the host-associated forms of *T. cristinae* are unlikely to have achieved species status by any criterion, as indicated by only a 60% barrier to gene flow at the premating level (Nosil *et al.* 2006) and a general lack of neutral mtDNA differentiation between adjacent populations on different hosts due to ongoing gene flow (Nosil *et al.* 2003). Thus these host forms represent either an ongoing speciation event or population divergence that has reached equilibrium. A major question is why has complete speciation not occurred? Further studies of more divergent species within this genus may shed light onto the ecological,

genetic or geographic factors driving the transition from a host race or ecotype to a species (Sandoval & Nosil 2005).

Parallels and contrasts with other orthopteroids

Orthopteroids have a long history as model systems in speciation research, and different groups exhibit patterns that both parallel and contrast those observed in *Timema* walking sticks. Here I aim to outline some well-studied groups, rather than to provide an exhaustive review. I first consider the relative roles of natural versus sexual selection, and then examine the evidence for reinforcement.

In *T. cristinae*, ecological divergence in host plant use promoted the evolution of sexual isolation. Divergence in host plant use has been shown to promote the evolution of sexual isolation in a few other nonorthopteran, herbivorous insect taxa (e.g., *Neochlamisus* leaf beetles: Funk 1998, Funk *et al.* 2002 for a review).

In contrast, ecological divergence does not seem critical for the evolution of reproductive isolation (and for sexual isolation in particular) in some Orthoptera. For example, closely related species of *Laupala* crickets on the Hawaiian Islands are ecologically similar and appear to differ primarily in calling song, a secondary sexual trait used in mate attraction (Mendelson & Shaw 2002, 2005). Differences between species in sexually-selected calling songs form the basis of sexual isolation between them, such that rapid speciation by sexual selection may have occurred. Another example of the evolution of sexual isolation by sexual selection comes from field crickets, where the key feature distinguishing the cryptic sister species *Gryllus texensis* and *G. rubens* is pulse rate, and the other conditions for speciation by sexual selection are met (Gray & Cade 2000).

An example where calling song may not be involved comes from montane grasshoppers in the genus *Melanopus* from the sky islands (Heald 1967) of western North America, where once again there is evidence for relatively rapid speciation (Knowles 2000). *Melanopus* species are quite morphologically similar, and differ primarily in male genitalia, a trait posited to be under sexual selection (a direct link between sexual isolation and genital divergence is yet to be established). Finally, divergence in mating signals among populations of the grasshopper *Chorthippus parallelus* is associated with some aspect of the colonization process (i.e., founder events), such that ecological divergence alone does not appear to drive the evolution of sexual isolation (Tregenza *et al.* 2000).

Perhaps even more striking than the examples above are cases of mosaic hybrid zones in grasshoppers and crickets. In these zones, closely related species do exhibit some habitat (i.e., ecological) specificity, yet reproductive isolation is not always strongly associated with ecological divergence.

Mosaic hybrid zones can be formed by long-distance dispersal into the previously unoccupied region between 2 advancing populations (Nichols & Hewitt 1994, Ibrahim *et al.* 1996). However, when a close correspondence between particular genotypes and discernible environmental patches is observed, this explanation is implausible (Barton & Hewitt 1985). In such situations, the patchiness of these zones is more readily attributed to some combination of active habitat preference on the one hand and divergent natural selection on the other.

Several such habitat-associated mosaic zones have been documented between species of grasshoppers or crickets (e.g., *Gryllus*, Rand & Harrison 1989; reviewed in Nosil *et al.* 2005a), but reproductive isolation is not always clearly ecologically driven. For example, in the mosaic hybrid zone between *Allonemobius fasciatus* and *A. socius*, genetic structure is associated with patches of different temperature

(Howard 1986). However, the main reproductive barrier between these species appears to be conspecific sperm precedence, rather than sexual isolation (Howard *et al.* 1998). In some cases there is a partial role for ecology, but other factors also play an apparent role. In the mosaic hybrid zone between *Chorthippus brunneus* and *C. jacobsi*, genotypic composition is significantly, but weakly, associated with patches of different vegetation type and local habitat makes only a small contribution in explaining deviations in calling song and stridulatory peg number from clinal expectation (Bridle *et al.* 2001, Bridle & Butlin 2002). These examples suggest that ecological divergence is not critical for the evolution of sexual isolation between closely-related grasshopper and cricket taxa, at least for the taxa studied.

Why do some groups exhibit strong resource-associated (i.e., ecologically-based) divergence, whereas other groups diversify with less ecological differentiation? Two potential explanations concern the feeding lifestyle of different groups and the types of traits used in mate choice. An explanation for differences among groups is offered by the 'grazer-parasite hypothesis'; difference among groups could be a function of how closely associated the insects are with their food resources (reviewed by Thompson 1988). In 'grazer' species, individuals must move between two or more plant individuals to complete their development. 'Parasites' are species where individuals complete development on a single plant individual. It might be expected that 'parasites' (such as *Timema*) are more prone to speciation via resource-associated divergence, due to their closer association with particular food items. Thus perhaps many Orthoptera are not tied closely enough to a specific food resource to have the evolution of reproductive isolation closely associated with resource divergence. Notably, recent molecular studies have shown genetic lineages associated with host plants or ecological features (i.e., host-associated divergence) even in polyphagous, 'generalist' grasshoppers (Dopman *et al.* 2002, VanDyke *et al.* 2004, Sword *et al.* 2005). Further studies examining the strength of association with resources in relation to the causes of speciation will likely prove insightful. The prediction is that groups more closely associated with their food resources will be more likely to evolve sexual isolation via resource-associated natural selection.

Another explanation for differences among groups considers the types of traits involved. Once again a clear prediction can be made: the evolution of sexual isolation by natural selection is only expected when traits conferring sexual isolation are under natural selection (i.e., are inherently 'ecological'). Calling song is inherently sexual (being used to attract mates), such that speciation in singing insects need not involve ecological divergence (a similar argument could be made for other sexual characters such as genitalia). Interestingly though, some models of speciation via sexual selection include a role for natural selection (Lande & Kirkpatrick 1988, Schluter 2000). For example, the 'sensory drive' hypothesis predicts that mating signals will evolve in correlation with aspects of the environment (Endler 1992, Boughman 2002). Calling song in birds and frogs has been shown to be affected by the ecological environment (Morton 1975, Ryan *et al.* 1990, Slabbekoorn & Smith 2002, Seddon 2005) and it would be interesting to consider the role of ecology in affecting the evolution of song in singing Orthoptera. For example, singing Orthoptera need to avoid predation such that predation regimes could affect the evolution of song. Moreover, different types of habitats may vary in their sound transmission properties, imposing habitat-specific selection on signaling systems. Most of the examples discussed exhibit contrasting results to those observed in *T. cristinae*, and some potential explanations have been

outlined. However, there are some parallels as evidenced by the examples of host-associated genetic divergence and habitat-specific differentiation in hybrid zones.

I now turn my attention to reproductive character displacement and reinforcement. As noted by Howard (1993), reproductive character displacement among the singing Orthoptera has played a large role in arguments over the likelihood and importance of reinforcement. Walker (1974) noted that there were few solid cases of reproductive character displacement in male calling song in Orthoptera, but also pointed out that few studies were designed to test for such a pattern. Moreover, displacement could occur in female preferences even if male traits are not displaced, *i.e.*, reinforcement occurs but is detectable only if female preferences are reexamined (*e.g.*, Hobel & Gerhardt 2003). Otte (1989) reported several cases of male calling song displacement in sword-tail crickets of the Hawaiian islands. Yang and Gerhardt (2006) report similar patterns of song displacement in the southern wood cricket (*Gryllus fultoni*). In contrast, Veech *et al.* (1996) found no evidence for character displacement in a detailed study of male song in ground crickets (see also Gregory *et al.* 1998). Likewise, studies of field crickets (*Gryllus texensis* and *Gryllus rubens*) found no evidence for reproductive character displacement (Gray & Cade 2000, Izzo & Gray 2004). Thus the evidence for reinforcement in singing Orthoptera appears equivocal.

The results from *T. cristinae* suggest that the likelihood and effects of reinforcement are highly dependent on relative population size and migration rates. Variation in the occurrence of reinforcement might be explained by differences among groups in habitat structure (*i.e.*, geographic arrangement of populations), demography, and dispersal characteristics.

Summary

In *T. cristinae*, ecological divergence and reinforcement interact to drive the evolution of sexual isolation. In contrast, gene flow between populations can erode divergence such that both selection and gene flow are important determinants of the evolution of sexual isolation. As discussed, similar and contrasting patterns can be seen in other orthopteroids. This variation among groups might reflect the degree to which different groups are intimately associated with their food resources, the types of traits used in mate choice, and how the geographic arrangement of populations affects the opportunity for reinforcement.

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References

- Barton N.H., Hewitt G.M. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* 16: 113-148.
- Barton N.H., Turelli M. 1991. Natural and sexual selection on many loci. *Genetics* 127: 229-225.
- Boughman J.W. 2002. How sensory drive can promote speciation. *Trends in Ecology and Evolution* 10: 1-7.
- Bridle J.R., Baird S.J.E., Butlin R.K. 2001. Spatial structure and habitat variation in a grasshopper hybrid zone. *Evolution* 55: 1832-1843.
- Bridle J.R., Butlin R.K. 2002. Mating signal variation and bimodality in a mosaic hybrid zone between *Chorthippus* grasshopper species. *Evolution* 56: 1184-1198.
- Butlin R.K. 1995. Reinforcement: an idea evolving. *Trends in Ecology and Evolution* 10: 432-433.
- Cain M.L., Andreassen V., Howard D.J. 1999. Reinforcing selection is effective under a relatively broad set of conditions in a mosaic hybrid zone. *Evolution* 53: 1343-1353.
- Coyne J.A., Orr H.A. 2004. *Speciation*. Sinauer Associates, Sunderland, MA.
- Crespi B.J., Sandoval C.P. 2000. Phylogenetic evidence for the evolution of ecological specialization in *Timema* walking sticks. *Journal of Evolutionary Biology* 13: 249-262.
- Dobzhansky T. 1937. *Genetics and the Origin of Species*. Columbia University Press, New York.
- Dopman E.B., Sword G.A., Hillis D.M. 2002. The importance of the ontogenetic niche in resource-associated divergence: evidence from a generalist grasshopper. *Evolution* 56: 731-740.
- Endler J.A. 1992. Signals, signal conditions, and the direction of evolution. *American Naturalist* 139: 125-153.
- Funk D.J. 1998. Isolating a role for natural selection in speciation: host adaptation and sexual isolation in *Neochlamisus bebbianae* leaf beetles. *Evolution* 52: 1744-1759.
- Funk D.J., Filchak K.E., Feder J.L. 2002. Herbivorous insects: model systems for the comparative study of speciation ecology. *Genetica* 116: 251-267.
- Gray D.A., Cade W.H. 2000. Sexual selection and speciation in field crickets. *Proceedings of the National Academy of Sciences USA* 97: 14449-14454.
- Gregory P.G., Remmenga M.D., Howard D.J. 1998. Patterns of mating between two closely related ground crickets are not influenced by sympatry. *Entomologia Experimentalis et Applicata* 87: 263-270.
- Heald W.F. 1967. Sky Island. Van Nostrand, Princeton, N.J.
- Higgie M., Chenoweth S., Blows M.W. 2000. Natural selection and the reinforcement of mate recognition. *Science* 290: 519-521.
- Hobel G., Gerhardt H.C. 2003. Reproductive character displacement in the acoustic communication system of green tree frogs (*Hyla cinerea*). *Evolution* 57: 894-904.
- Howard D.J. 1986. A zone of overlap and hybridization between two ground cricket species. *Evolution* 40: 34-43.
- Howard D. J. 1993. Reinforcement: origins, dynamics and fate of an evolutionary hypothesis, pp. 46-49. In: Harrison R.G. (Ed.) *Hybrid Zones and the Evolutionary Process*. Oxford University Press, New York.
- Howard D.J., Gregory P.G., Chu J.M., Cain M.L. 1998. Conspecific sperm precedence is an effective barrier to hybridization between closely related species. *Evolution* 52: 511-516.
- Ibrahim K. M., Nichols R.A., Hewitt G.M. 1996. Spatial patterns of genetic variation generated by different forms of dispersal during range expansions. *Heredity* 77: 282-291.
- Izzo A.S., Gray D.A. 2004. Cricket song in sympatry: species specificity of song without reproductive character displacement in *Gryllus rubens*. *Annals of the Entomological Society of America* 97: 831-837.
- Jiggins C.D., Naisbit R.E., Coe R.L., Mallet J. 2001. Reproductive isolation caused by colour pattern mimicry. *Nature* 411: 302-305.
- Kirkpatrick, M. 2000. Reinforcement and divergence under assortative mating. *Proceedings of the Royal Society of London (B)* 267: 1649-1655.
- Kirkpatrick M. 2001. Reinforcement during ecological speciation. *Proceedings of the Royal Society of London (B)* 268: 1259-1263.

- Knowles L.L. 2000. Tests of Pleistocene speciation in montane grasshoppers (genus *Melanoplus*) from the sky islands of western North America. *Evolution* 54: 1337-1348.
- Lande R., Kirkpatrick M. 1988. Ecological speciation by sexual selection. *Journal of Theoretical Biology* 133: 85-98.
- Mayr E. 1947. Ecological factors in speciation. *Evolution* 1: 263-288.
- Mayr E. 1963. *Animal Species and Evolution*. Harvard University Press, Cambridge.
- Mendelson T.C., Shaw K.L. 2002. Genetic and behavioral components of the cryptic species boundary between *Laupala cerasina* and *L. kohalensis* (Orthoptera: Gryllidae). *Genetica* 116: 301-310.
- Mendelson T.C., Shaw K.L. 2005. Sexual behaviour: Rapid speciation in an arthropod. *Nature* 433: 375-376.
- Morton E.S. 1975. Ecological sources of selection on avian sounds. *American Naturalist* 109: 17-34.
- Nichols R. A., Hewitt G.M. 1994. The genetic consequences of long distance dispersal during colonization. *Heredity* 72: 312-317.
- Noor M.A.F. 1995. Speciation driven by natural selection in *Drosophila*. *Nature* 375: 674-675.
- Noor M. 1999. Reinforcement and other consequences of sympatry. *Heredity* 83: 503-508.
- Nosil P. 2004. Reproductive isolation caused by visual predation on migrants between divergent environments. *Proceedings of the Royal Society of London B* 271: 1521-1528.
- Nosil P., 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., Crespi B.J. 2006. Ecological divergence promotes the evolution of cryptic reproductive isolation. *Proceedings of the Royal Society of London B* 273: 991-997.
- Nosil P., Crespi B.J., Sandoval C.P. 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature* 417: 441-443.
- Nosil P., Crespi B.J., Sandoval C.P. 2003. Reproductive isolation driven by the combined effects of ecological adaptation and reinforcement. *Proceedings of the Royal Society of London B* 270: 1911-1918.
- Nosil P., Vines T.H., Funk D.J. 2005a. Perspective: Reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* 59: 705-719.
- Nosil P., Crespi B.J., Sandoval C.P. 2006. The evolution of host preference in allopatric versus parapatric populations of *T. cristinae* walking sticks. *Journal of Evolutionary Biology* 19: 929-942.
- Otte D. 1989. Speciation in Hawaiian crickets, pp. 482-526. In: Otte D., Endler J.A. (Eds) *Speciation and its Consequences*. Sinauer, Sunderland, MA.
- Peterson M.A., Honchak B.M., Locke S.E., Beeman T.E., Mendoza J., Green J., Buckingham K.J., White M.A., Monsen K.J. 2005. Relative abundance and the species-specific reinforcement of male mating preference in the *Chrysochus* (Coleoptera: Chrysomelidae) hybrid zone. *Evolution* 59: 2639-2655.
- Rand D.M., Harrison R.M. 1989. Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. *Evolution* 43: 432-449.
- Rundle H.D., Schluter D. 1998. Reinforcement of stickleback mating preferences: sympatry breeds contempt. *Evolution* 52: 200-208.
- Rundle H.D., Nagel L., Boughman J.W., Schluter D. 2000. Natural selection and parallel speciation in sympatric sticklebacks. *Science* 287: 306-308.
- Rundle H.D., Nosil P. 2005. Ecological speciation. *Ecology Letters* 8: 336-352.
- Ryan M.J., Crocroft R.B., Wilczynski W. 1990. The role of environmental selection in intraspecific divergence of mate recognition signals in the cricket frog, *Acris crepitans*. *Evolution* 44: 1869-1872.
- Saetre G. P., Moum T., Bures S., Kral M., Adamjan M., Moreno J. 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. *Nature* 387: 589-592.
- 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*. Ph.D. Thesis. University of California, Santa Barbara.
- Sandoval C.P. 1994a. The effects of relative geographic scales of gene flow and selection on morph frequencies in the walking stick *Timema cristinae*. *Evolution* 48: 1866-1879.
- Sandoval C. P. 1994b. Differential visual predation on morphs of *Timema cristinae* (Phasmatodeae: Timemidae) and its consequences for host range. *Biological Journal of Linnean Society* 52: 341-356.
- Sandoval C.P., 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. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
- Seddon N. 2005. Ecological adaptation and species recognition drives vocal evolution in neotropical suboscine birds. *Evolution* 59: 200-215.
- Servedio M. 2001. Beyond reinforcement: the evolution of premating isolation by direct selection on preferences and postmating, prezygotic incompatibilities. *Evolution* 55: 1909-1920.
- Servedio M.R., Kirkpatrick M. 1997. The effects of gene flow on reinforcement. *Evolution* 51: 1764-1772.
- Servedio M., Noor M. 2003. The role of reinforcement in speciation: theory and data. *Annual Review Ecological Systematics* 34: 339-364.
- Slabbekoom H., Smith T.B. 2002. Habitat-dependent song divergence in the little greenbul: an analysis of selection pressures on acoustic signals. *Evolution* 56: 1849-1858.
- Slatkin M. 1987. Gene flow and the geographic structure of natural populations. *Science* 236: 787-792.
- Sword G.A., Joern A., Senior L.B. 2005. Host plant-associated genetic differentiation in the snakeweed grasshopper, *Hesperotettix viridis* (Orthoptera: Acrididae). *Molecular Ecology* 14: 2197-2205.
- Thompson J.N. 1988. Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. *Entomologia Experimentalis Applicata* 47:3-14.
- Tregenza T., Pritchard V.L., Butlin R.K. 2000. The origins of premating reproductive isolation: testing hypotheses in the grasshopper *Chorthippus parallelus*. *Evolution* 54: 1687-1698.
- VanDyke K.A., Lockwood J.A., Kazmer D.J. 2004. Association of genetic lineages with ecological features in a polyphagous montane grasshopper species. *Journal of Orthoptera Research* 13: 205-209.
- Veech J.A., Benedix J.H., Howard D.J. 1996. Lack of calling song displacement between two closely related ground crickets. *Evolution* 50: 1982-1989.
- Waage J.K. 1979. Reproductive character displacement in *Calopteryx* (Odonata: Calopterygidae). *Evolution* 33: 104-116.
- Walker T.J. 1974. Character displacement and acoustic insects. *American Zoologist* 14: 1137-1150.
- Yang Y., Gerhardt H.C. 2006. Divergence in calling song between sympatric and allopatric populations of the southern wood cricket *Gryllus fultoni* (Orthoptera: Gryllidae). *Journal of Evolutionary Biology* 19: 459-472.