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Geographic variation in acoustic communication: reproductive character displacement and speciation

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ABSTRACT

Background: Reproductive character displacement is a geographical pattern in which mate-attracting signals, preferences or both differ more in areas of sympatry than in areas of allopatry.

Questions: What selective forces drive reproductive character displacement? What are its consequences?

Methods: Reproductive character displacement in mate-attracting signals and/or female preferences are described for pairs of closely related field crickets (*Gryllus*), periodical cicadas (*Magicicada*), chorus and treefrogs (*Pseudacris* and *Hyla*), and tinkerbirds (*Pogoniulus*). These examples were characterized by data from multiple populations and evidence regarding the efficacy of sympatric divergence in signals or preferences.

Conclusions: Whereas selection resulted in changes that reduce the production of genetically or behaviourally unfit hybrids in field crickets, chorus frogs, and green and grey treefrogs, reproductive character displacement in periodical cicadas and tinkerbirds was most likely driven by masking interference or mistakes in the identification of territorial rivals. Reproductive character displacement may not only accentuate or preserve divergence that arises in allopatry but can result in cascades of speciation if assortative mating arises between displaced and non-displaced populations.

Keywords: acoustic communication, auditory masking, cascades of speciation, geographical variation, sexual selection.

INTRODUCTION

Mutation, drift, and selection cause evolutionary change in mate-attracting acoustic signals and selective responses to these signals. Divergence in these traits in different groups of populations that experience restricted or no gene flow can lead to speciation. Assessing the taxonomic status of geographically isolated populations is problematic and arbitrary, but in areas where closely related taxa co-exist, assortative mating based on differences in acoustic signals is a hallmark of speciation.

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Communities of species with distinct communication systems are not necessarily a product of prior interactions between species. Differences arising in allopatry between closely related species may be sufficient to ensure that few, if any, mating mistakes occur when overlaps in distributions are re-established (e.g. Littlejohn, 1993). If, however, divergence in ecological requirements and mate-choice systems is insufficient to allow such non-interactive sympatry, then a range of outcomes can occur in zones of re-established contact, including: (1) replacement of one taxon by the other; (2) 'stable' hybrid zones; (3) reproductive character displacement; and (4) fusion. Many theoretical papers predict such multiple outcomes, and population size, gene flow, and ecological gradients are major determinants (e.g. Liou and Price, 1994; Servedio and Kirkpatrick, 1997; Servedio and Noor, 2003; Goldberg and Lande, 2006).

In agreement with Pfennig and Pfennig (2009), I consider reproductive character displacement as a geographic pattern that can be caused by multiple processes. Reinforcement is usually considered the predominant process and is often characterized as natural selection for species recognition (Coyne and Orr, 2004). In my view, this term has been muddled by a hodgepodge of definitions – not to mention its predominant use as a term referring to a psychological phenomenon – and by the difficulty of applying any of the many species concepts to transitional states (e.g. Mendelson and Shaw, 2012). It is my view that sexual selection is the primary process that affects communication systems and that species recognition is one possible consequence (e.g. Gerhardt and Huber, 2002; Mendelson and Shaw, 2012). Differences in some particular acoustic trait(s) may distinguish syntopically occurring species that rarely mismate, but assuming that these differences evolved for species recognition is often unwarranted.

Reinforcement also depends on hybrid genetic incompatibility or other forms of hybrid unfitness, yet interactions between species that never mismate and by-products of ecological adaptations can also have significant effects on communication systems (Gerhardt and Huber, 2002; Coyne and Orr, 2004; Pfennig and Pfennig, 2009; Hoskin and Higgie, 2010; Nosil and Hohenlohe, 2012). Geographic variation in communication systems is most likely to arise as an interaction between sexual selection and a variety of constraints (e.g. acoustically orienting predators and parasitoids, species with masking signals, habitat acoustics) imposed on such selection in different parts of the geographic distribution of a species. Genetic drift is also likely to play an important role in some systems (e.g. Campbell *et al.*, 2010).

Here I review examples of robust demonstrations of reproductive character displacement in acoustic communication systems where there is little doubt about the species status of the interacting taxa. There are few examples because I only consider studies that: (1) have sampled multiple populations in areas of allopatry and sympatry; and (2) have tested the selectivity of receivers (potential mates or territorial rivals). These tests must have shown that acoustic differences observed between sympatric and allopatric areas are biologically meaningful or that there are significant differences in receiver selectivity between such areas. Because the populations are the prime sampling units, comparing data from only a few populations weakens claims about reproductive character displacement and may fail to reveal interesting patterns of geographic variation within and between areas of allopatry and sympatry. Gabor and Ryan (2001) make the same point in discussing reproductive character displacement in visual mate choice in mollies.

The paucity of good examples suggests that reproductive character displacement is uncommon to rare, and I argue that there are two general reasons for this state of affairs. First, reproductive character displacement is expected to be uncommon for a variety of theoretical reasons. Second, there are practical difficulties of conducting these studies, especially with regard to behavioural testing of receivers from multiple populations. Hence there are very few studies that can confidently test for reproductive character displacement and its causes. In addition to discussions of studies that robustly demonstrate reproductive character displacement, I will summarize the results of a few studies that meet these criteria yet failed to demonstrate reproductive character displacement. A third, more specific reason is that receivers of some taxa (especially birds and mammals) use other sensory modalities in addition to audition in close-range mate choice. I will explain how this factor and others may account for the rarity of reproductive character displacement in birds and the absence, to my knowledge, of any good example in mammals.

ADVANTAGES OF STUDYING ACOUSTIC COMMUNICATION

Acoustic communication systems have several advantages for the study of geographical variation. First, many nocturnally courting species – especially acoustic insects and frogs – use this modality as the sole basis for mate choice (Gerhardt and Huber, 2002). In many birds, sounds play a major role in territorial defence, especially in areas of dense vegetation, but visual and acoustic cues operating at close range probably minimize mating mistakes. Second, acoustic signals readily indicate the location of the animals, thus facilitating sampling of multiple populations. Third, signals are relatively easy to record, usually at distances where receivers are likely to assess signals prior to initiating a response. Fourth, high-fidelity playbacks of animal signals often elicit unequivocal responses from receivers in the form of acoustic responses or positive phonotaxis (Gerhardt and Huber, 2002). Such experiments also have the advantage of much greater stimulus control than experiments involving visual or chemical cues. Fifth, acoustic signals are easy to synthesize, allowing researchers to vary particular properties in order to test whether statistically significant differences among individuals or populations are also behaviourally significant. Playbacks can also estimate the multivariate basis of mate choice by receivers and have revealed interactions between different, behaviourally relevant properties (e.g. Gerhardt, 1995; Ryan and Rand, 2003; Higgins and Waugaman, 2004; Castellano and Rosso, 2007; Gerhardt and Brooks, 2009). Recent studies show great promise in elucidating the genetic bases of acoustic signal production and preferences, even given their polygenic basis (e.g. Oh *et al.*, 2012).

EXAMPLES OF REPRODUCTIVE CHARACTER DISPLACEMENT

Field crickets

As indicated in Table 1, no evidence or equivocal evidence for reproductive character displacement was reported in five pairs of orthopteran species. Sampling ranged from modest to extensive, and one problem – which has plagued many such studies – is that the taxonomic status of individuals from different populations was unresolved, in part because of hybridization. The calling songs of the two *Teleogryllus* species are highly distinctive and allopatric females were as selective as sympatric ones, suggesting non-interactive attainment of sympatry.

Reproductive character displacement was documented both in the signals and preferences of the wood cricket *Gryllus fultoni* where it overlaps or occurs parapatrically with *G. vernalis* in an area between eastern Kansas and west of the Appalachian Mountains

Table 1. Negative or equivocal studies of reproductive character displacement (RCD)

Taxa	Results	Reference
Field crickets		
Teleogryllus commodus, *T. oceanicus*	No RCD calls or preferences	Hill *et al.* (1972)
Gryllus texensis, *G. rubens*	No RCD calls; preference and taxonomy equivocal	Higgins and Waugaman (2004)
Ground crickets		
Allonebomius fasciatus, *A. socius*	No RCD calls	Veech *et al.* (1996)
Sword-tailed crickets		
Laupala species complex	RCD equivocal because taxonomy is equivocal	Otte (1989), Shaw (1996)
Grasshoppers		
Chorthippus parallelus subspecies with call differences, genetic incompatibility	No RCD calls; equivocal evidence of female preference	Butlin (1989)
Cricket frogs		
Acris crepitans, *A. blanchardii*	No RCD calls	Ryan and Wilczynski (1991)
Narrow-mouthed toads		
Gastrophryne carolinensis, *G. olivacea*	Equivocal RCD calls	Loftus-Hills and Littlejohn (1992)
Singing mice		
Scotinomys teguina, *S. xerampelinus*	No RCD calls	Campbell *et al.* (2010)

(Fig. 1). The distribution of *G. fultoni* is more extensive, with large areas of allopatry *vis-à-vis G. vernalis* in the southeastern and middle-Atlantic USA and lower Gulf Coast. Field recordings of calling songs were analysed from 13 populations of *G. fultoni* [7 from allopatric areas and 6 from sympatric areas (Jang and Gerhardt, 2006a)], and from 11 populations of *G. vernalis* [3 from allopatric areas and 8 from sympatric areas (Jang *et al.*, 2007)] (Fig. 1). The two species have different microhabitat preferences in areas of sympatry, but are nevertheless found in close (and almost certainly audible) proximity. Any hybrids that survive and reach sexual maturity could thus be disfavoured by ecological selection; genetic incompatibility was demonstrated in laboratory crosses between the two species (Jang and Gerhardt, 2006a).

In *G. fultoni*, weak clinal variation in chirp and pulse rate (higher in the west in both properties; higher in the north in pulse rate) was detected, mostly within allopatric areas; nevertheless, this variation did not explain the pattern of reproductive character displacement that was detected in pulse rate at high temperatures and in chirp rate over a wide range of temperatures (Fig. 2). Values of these properties were intermediate in 'near' allopatric populations compared with 'far' allopatric and sympatric populations, suggesting gene flow. Four properties of the calling song in *G. vernalis* showed limited geographic variation and no evidence of sympatric divergence, perhaps because few populations were located in allopatric areas despite extensive surveys (Jang *et al.*, 2007). Analysis of the songs of the progeny of field-caught crickets of both species corroborated the geographic differences revealed by field recordings.

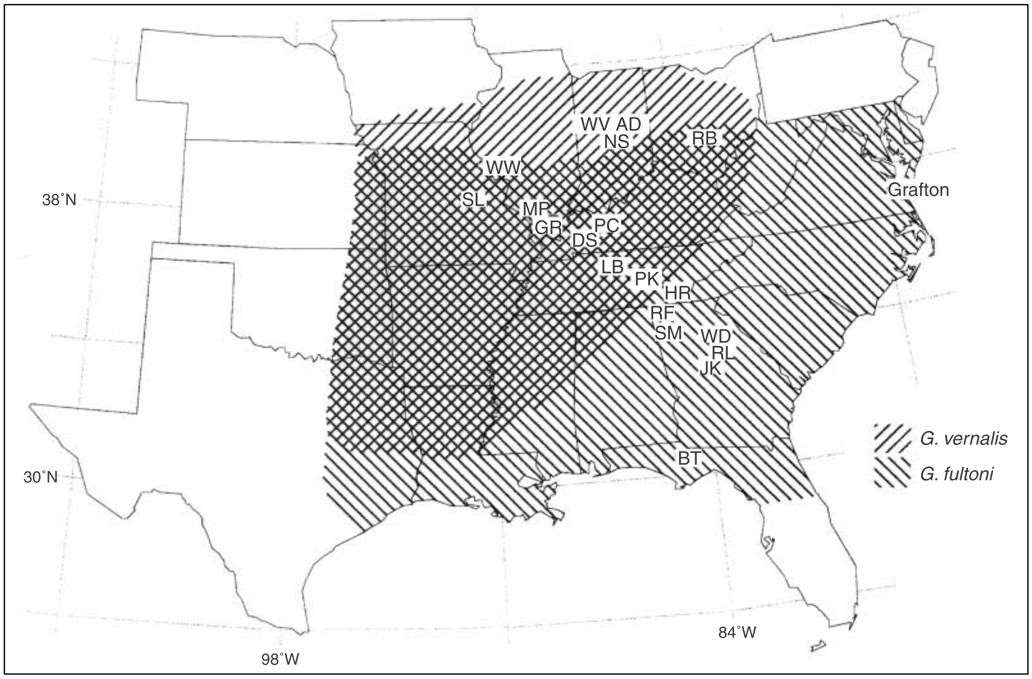


Fig. 1. Geographical distributions of two species of wood crickets. Letters show locations where calling songs were recorded [exact locations in Jang and Gerhardt (2006a), Jang *et al.* (2006b)]. Grafton indicates the source call data analysed by Doherty and Callos (1991). Females were collected at DS, JK, RL, and RF; their female offspring were raised in the laboratory and tested in playback experiments. Modified from Jang and Gerhardt (2006a).

The biological significance of calling-song differences was explored using single-speaker and two-stimulus, forced-choice playback experiments that employed synthetic calls (Jang and Gerhardt, 2006b). The acoustic properties of these sounds were modelled after the pattern of geographic variation in *G. fultoni* as described above and estimated from recordings in 'far' allopatry, sympatry, and in three intermediate populations. A synthetic call modelled after a typical male of *G. vernalis* was also tested. Females of *G. fultoni* were collected from some of the populations (see Fig. 1), representing populations in far allopatry (RL and JK), near allopatry (RF), and sympatry (DS). Offspring of these females were raised in the laboratory, and tested at about 23°C, which is near the middle of the normal range of calling temperature. In single-speaker tests, females from far allopatry were more likely to respond to the heterospecific signal and to signals from intermediate locations than were females from near allopatric and sympatric populations (Fig. 3a).

Although females from all three areas reliably chose the conspecific signal over the heterospecific one, females from sympatric and near allopatric areas were more likely to discriminate against songs typical of intermediate populations (in favour of a standard stimulus) than were females from far allopatric populations (Fig. 3b). Thus the pattern of reproductive character displacement documented in analyses of calling songs was roughly paralleled by the pattern of reproductive character displacement in female preference.

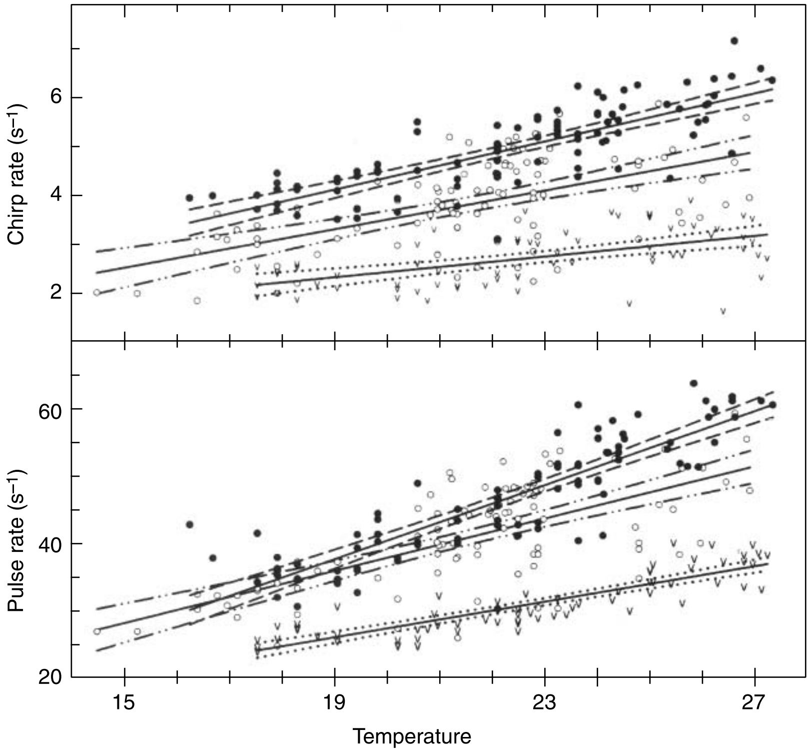


Fig. 2. Values of two properties of field-recorded calling songs as a function of temperature. Solid lines show the trends, which are bracketed by 95% confidence limits, coded as follows: *Gryllus fultoni* (allopatric: ○, –···–, $n = 99$; sympatric: ●, –––, $n = 92$); *G. vernalis* (v, ····). Modified from Jang and Gerhardt (2006a).

In summary, studies of these two wood crickets show geographic variation in both calling-song properties and female preferences in *G. fultoni*. Variation also occurred within areas of sympatry and allopatry, revealing weak clinal patterns, an asymmetric pattern of reproductive character displacement (absent in *G. vernalis*, at least judging from limited sampling in allopatric areas), and probable gene flow between sympatric and allopatric populations of *G. fultoni*. Note that merely testing females from one sympatric and one allopatric population with conspecific versus heterospecific songs would have failed to reveal the enhanced selectivity of females from sympatric and near allopatric areas.

Periodical cicadas

Periodical cicadas (genus *Magicicada*) have been subjects of intensive studies that provide evidence for allochronic speciation and reproductive character displacement in their chorus calls and preferences (Marshall and Cooley, 2000; Cooley *et al.*, 2001, 2006). Cooley *et al.* (2006) sampled 10 localities in the eastern USA, where they made chorus recordings and tested female responses to playbacks (Fig. 4). The species involved were *Magicicada tredecim* and *M. neotredecim*, 13-year cicadas with partially overlapping ranges, and *M. septendecim*, a 17-year cicada that served as a proxy for pre-contact *M. neotredecim*. The rationale for studying *M. septendecim* was its identification as the ancestral parent of *M. neotredecim*

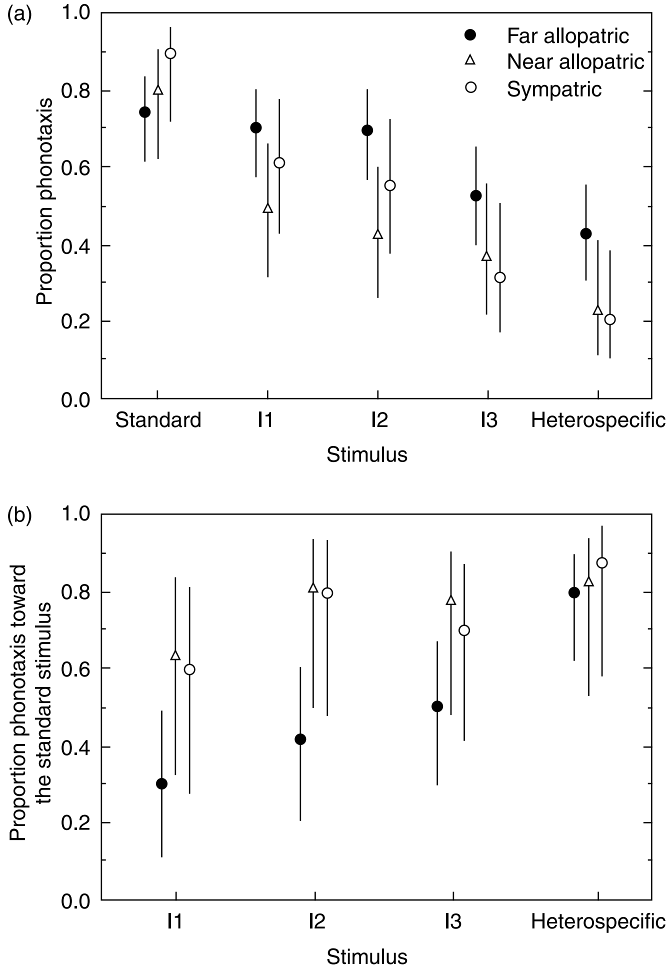


Fig. 3. Phonotactic responses and preferences in laboratory-reared females of *Gryllus fultoni*: (a) single-stimulus experiments; (b) two-stimulus preference tests. The standard (synthetic) stimulus had values typical of the female's population; the heterospecific stimulus had values typical of *G. vernalis*; I1, I2, and I3 had values intermediate between those typical of far allopatric and sympatric populations. In single-stimulus tests, the y-axis shows the proportion of females showing positive phonotaxis. In the two-stimulus tests, the y-axis shows the proportion of females choosing the standard stimulus. Error bars are 95% confidence intervals. The geographical sources of eggs from females where raised and tested are described in the text. Note that females from the far allopatric population were more likely to respond to and choose songs representative of intermediate populations than were females from sympatric and near-allopatric locations. Data from the two far allopatric populations were combined. Reproduced from Jang and Gerhardt (2006b) with kind permission from Springer Science + Business Media.

and the similarity of its calls and preferences to those of allopatric (with respect to *M. tredecim*) populations of *M. neotredecim*.

In addition to corroborating the reproductive character displacement in calls and preferences in *M. neotredecim*, Cooley *et al.* (2006) tested elements of an 'acoustic interference'

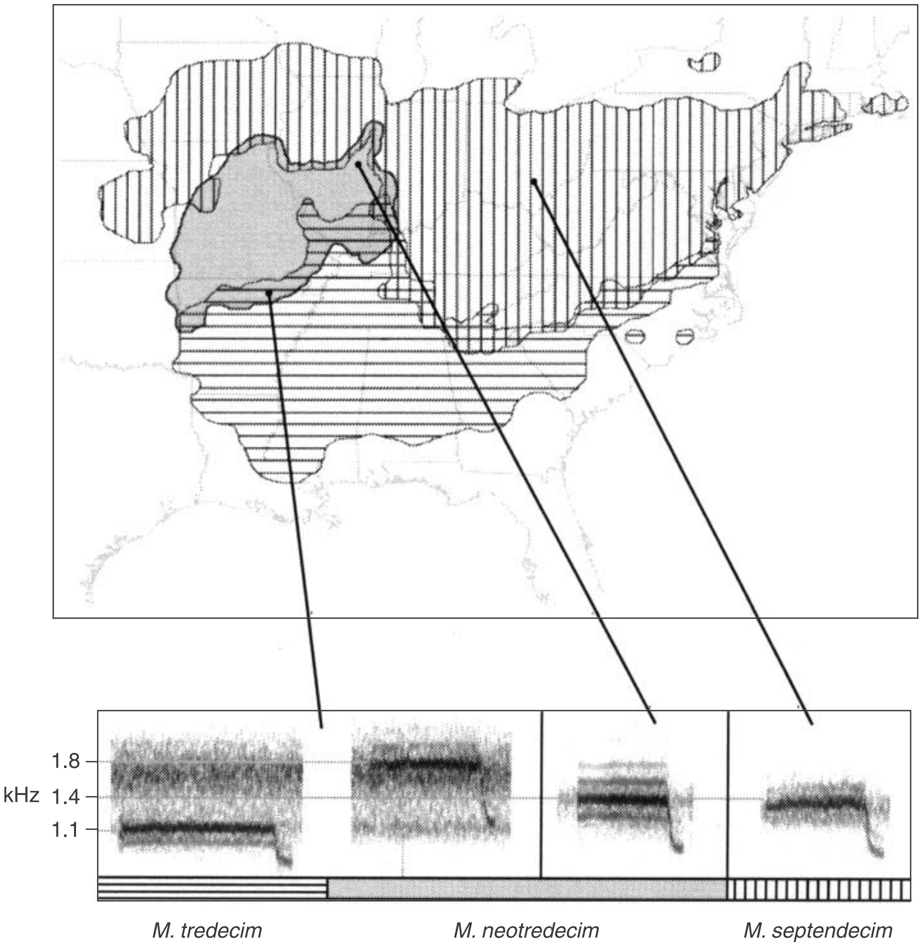


Fig. 4. The distributions of *Magicicada neotredecim* (shaded), *M. tredecim* (horizontal lines), and *M. septendecim* (vertical lines) in the eastern United States, with appended sonograms [graphs of song pitch (kHz) vs. time], for typical *M. septendecim* and *M. tredecim*, and for displaced and undisplaced *M. neotredecim*. The three species are largely parapatric. Reproduced from Cooley *et al.* (2006) with kind permission from John Wiley and Sons.

hypothesis that may explain the asymmetrical reproductive character displacement. In contrast to *M. neotredecim*, calls and preferences of *M. tredecim* show only a small amount of sympatric divergence in some broods. As shown in Fig. 4, the calls of the two species differ in frequency and contain a short downward modulation at the end that triggers female responses in the form of wing flicks. Shifts to higher-frequency calls and down-slurs in *M. neotredecim* in sympatric areas should reduce masking by the constant-frequency parts of *M. tredecim* calls; downward shifts in *M. tredecim* would be superfluous because the very brief terminal down slurs of that species would be free of overlap with either the main call or down-slurs of *M. neotredecim*. The interference hypothesis was bolstered by the results of experiments showing that females of *M. neotredecim* in sympatric areas shift their frequency preference in parallel with the male calls (Fig. 5). The results of masking experiments with *M. septendecim* were also supportive. Females subjected to chorus background noise of *M. tredecim* facultatively shifted their preference to higher frequencies, hence demonstrating that masking affects preferences. Presumably genetic variation underlying auditory mechanisms affecting preferences could have been subject to selection leading to an evolutionary change in sympatric *M. neotredecim* (Cooley *et al.*, 2006).

Although studies of the viability of laboratory crosses between *M. tredecim* and *M. neotredecim* revealed no genetic incompatibility, Cooley *et al.* (2006) argue that the reproductive character displacement is not completely explained by the acoustic interference hypothesis. While their arguments about hybrid sterility or ecological and behavioural deficits are plausible, there is no solid evidence for either explanation.

My view is that these results are significant because they provide a robust demonstration that acoustic interference alone can serve as a source of selection on acoustic communication systems. Comparable results have been presented for some frogs and birds (Amézquita *et al.*, 2006; Kirschel *et al.*, 2009a), although, as discussed below, the results of playback experiments are required to assess the influence of masking on receivers.

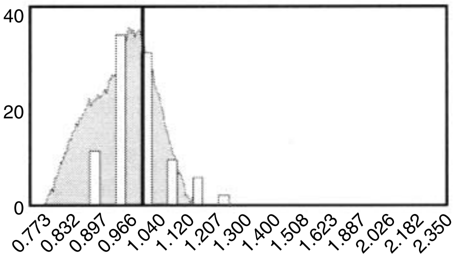
Anurans

Chorus frogs and 'cascades' of reproductive character displacement

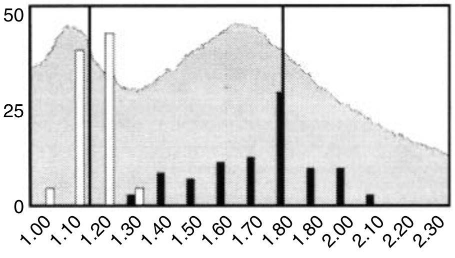
A classic example of reproductive character displacement involves chorus frogs in the southeastern USA. Fouquette (1975) demonstrated an asymmetric pattern of reproductive character displacement, mainly in the shift to higher pulse rate of the calls of *Pseudacris feriarum* in areas where it occurs with *P. nigrita* in a well-sampled north–south transect roughly following the border of Georgia and Alabama into the panhandle of Florida. Lemmon and Lemmon (2010) demonstrated the genetic incompatibility of hybrids and the relative unattractiveness of their calls. Lemmon (2009) sampled calls much more extensively than Fouquette, added data on a third species (*P. brimleyi*), and tested the phonotactic selectivity of females of *P. feriarum* from a sympatric and an allopatric population. The ranges of the widely distributed *P. feriarum* and *P. nigrita* have broad areas of overlap as well as extensive areas where they are allopatric with respect to each other. The range of *P. brimleyi* is overlapped entirely by that of one or the other of the two species, and there are a few areas where all three species not only co-exist but form mixed species breeding choruses (Fig. 6). Lemmon (2009) found that a second property, pulse number, also showed significant geographical variation in *P. feriarum*, especially in areas of overlap with *P. brimleyi*. More specifically, pulse number but not pulse rate in calls of *P. feriarum* increased in the three-species site (SC in Fig. 7). The reason may be that, while increasing pulse rate would cause divergence *vis-à-vis* pulse rate in *P. nigrita*, it would decrease the difference with respect to that of *P. brimleyi*.

Preference tests showed that higher proportions of females of *P. feriarum* from a population sympatric with *P. nigrita* chose the conspecific stimulus than did females from an allopatric population (Fig. 8). Choices of the non-displaced conspecific stimulus by such females was still high, but would probably decrease if a more realistic playback design had been used in which the intensity of the preferred call was reduced to reflect situations in which females first encounter heterospecific calling males (see next two sections). Lemmon (2009) argues that the preferences were probably not merely preferences for the calls of local

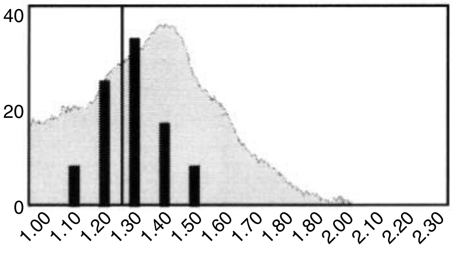
(a) Allopatric *M. tredecim*, Brood XXII



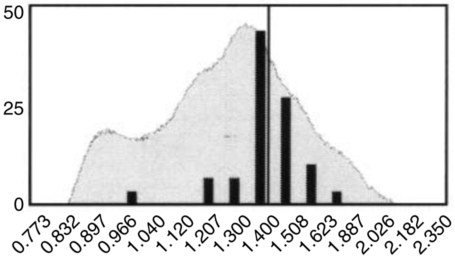
(b) Overlap Zone, Brood XIX



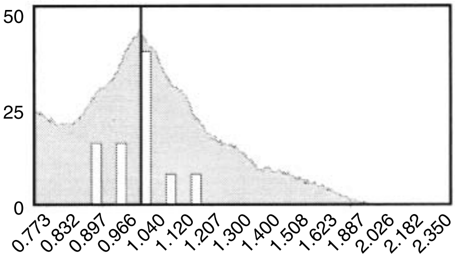
(c) Allopatric *M. neotredecim*, Brood XIX



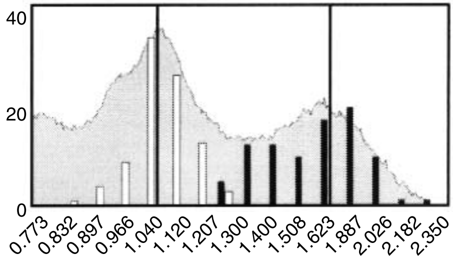
(d) *M. septendecim*, Brood VII



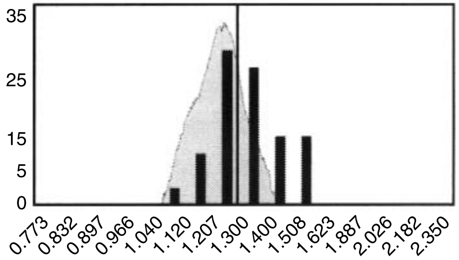
(e) Allopatric *M. tredecim*, Brood XXIII



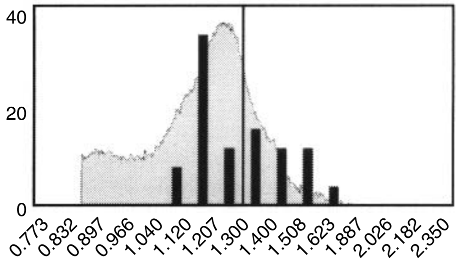
(f) Overlap Zone, Brood XXIII



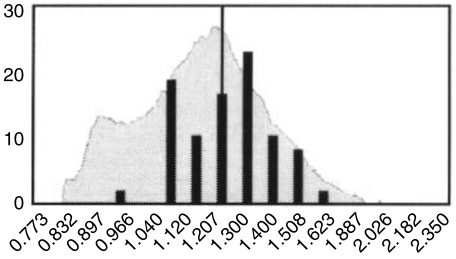
(g) Allopatric *M. neotredecim*, Brood XXIII



(h) *M. septendecim*, Brood VIII



(i) *M. septendecim*, Brood IX



males because females from the allopatric population preferred the calls of conspecifics from the sympatric population to those of local males.

This last result is consistent with the idea that reproductive character displacement has the potential to promote 'cascades' of speciation (Howard, 1993; Hoskin and Higgie, 2010; Nosil and Hohenlohe, 2012), in which assortative mating and potential reproductive isolation occur on the basis of differences in the signals of displaced and non-displaced populations. Two examples from studies of hylid frogs in Australia are also consistent with this hypothesis. The pulse rates of *Litoria verreauxii* and *L. ewingii* in western sympatry differ by about 60% (Littlejohn, 1993), which is more than sufficient for premating isolation (Littlejohn and Loftus-Hills, 1968; Loftus-Hills and Littlejohn, 1971) (Fig. 9). Furthermore, the depth of modulation of notes is often greatly reduced in calls of *L. verreauxii* in sympatry, sometimes to the extent that notes are no longer pulsed (Littlejohn, 1965; Gerhardt and Davis, 1988). The 'cascade' hypothesis is supported directly by the fact that females from sympatric populations prefer the calls of local males to those of conspecific males from remote allopatric areas as well as the calls of *L. ewingii* (Littlejohn and Loftus-Hills, 1968). In *Litoria genimaculata*, differential divergence in calls in two different nearby contact zones between genetically incompatible lineages in northeastern Queensland results in behaviourally significant call differences capable of mediating lineage-assortative mating in the contact zones (Hoskin *et al.*, 2005). The greater call divergence in the northern contact zone, where there is a significant shift in call frequency correlated with a dramatic decrease in male body size, is also sufficient to promote call discrimination against these 'displaced' calls by females from allopatric populations of the same lineage.

In my view, both of these studies can be improved by additional population sampling of calls and female selectivity in allopatric areas. These data are crucial for calibrating the degree to which divergence caused by species (lineage) interactions departs from geographical differences arising from other processes. As shown by Lemmon's study of chorus frogs and two other anuran species discussed below, significant differences in communication systems can be expected in both allopatric and sympatric areas. Indeed, sympatry or allopatry *vis-à-vis L. ewingii* does not explain significant call differences among some populations of *L. verreauxii alpina* (Smith *et al.*, 2003).

Green and barking treefrogs

Despite distinctive differences in morphology, advertisement calls, and calling-site preferences, the broadly sympatric green (*Hyla cinerea*) and barking treefrog (*H. gratiosa*) are highly genetically compatible, and hybrids are regularly encountered (Gerhardt *et al.*, 1980) (Fig. 10). Moreover, introgressive hybridization was documented in a fish hatchery near Auburn, Alabama over a period of more than 20 years (Lamb and Avise, 1985; Schlefer *et al.*, 1986). The most plausible explanation is that green treefrog males often adopt satellite behaviour,

Fig. 5. Histograms of female weighted average preferences superimposed on acoustical power spectra of male chorus pitch for allopatric populations and for populations within the *M. tredecim*/*M. neotredecim* overlap zone. *Magicicada tredecim* female preferences are indicated by open bars, *M. neotredecim* and *M. septendecim* by solid bars; mean population female preferences are indicated by a vertical line. Logarithmic power spectra of male chorus pitch (shaded) are indications of relative acoustical power scaled in decibels. Reproduced from Cooley *et al.* (2006) with kind permission from John Wiley and Sons.

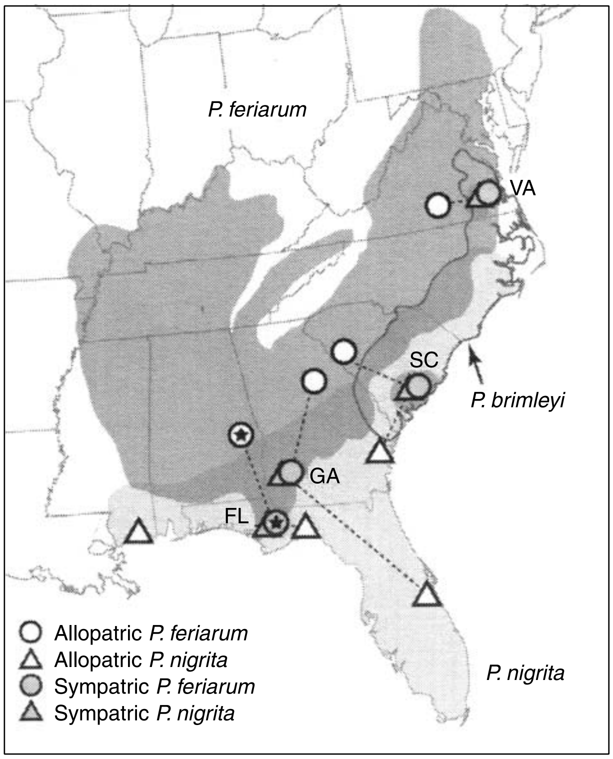


Fig. 6. Distributions of *Pseudacris feriarum* and *P. nigrita* in the southeastern United States and populations sampled in the study by Lemmon (2009). Call transects are indicated by dashed lines and State abbreviations. The distribution of *P. brimleyi* is delineated by a solid grey line. Female preference experiments were conducted in the two populations marked with a black star. Modified from Lemmon (2009). Reproduced with kind permission from John Wiley and Sons.

attempting to intercept females moving towards nearby calling males (Perrill *et al.*, 1978). Males usually call from elevated sites on emergent vegetation around a pond or lake (Oldham and Gerhardt, 1975), but the lack of such vegetation forced males to call from the ground at the fish hatchery. This increased the chances that calling males and their satellites would clasp females of *H. gratiosa* as they moved to conspecific males, which call from floating positions in the pond. Analysis of the parentage of hybrids and backcrosses at the Auburn fish hatchery, where male green treefrogs call on the mowed edges of ponds, confirmed this expectation (Lamb and Avise, 1985).

The spectral properties of the calls of the two species are well differentiated, and the high level of selectivity of females of both species in tests with pre-recorded calls (including those of putative hybrids) was demonstrated some time ago by playback experiments (Gerhardt, 1974a; Oldham and Gerhardt, 1975). Höbel and Gerhardt (2003) sampled calls of *H. cinerea* from four localities in allopatry and six in geographical sympatry; in two of the latter populations, males of the two species called at the same time and place (Fig. 10). There was modest geographic variation in the frequency of the lower spectral peak consistent with reproductive character displacement, but some differences between sympatric populations were as large as the largest differences between sympatric and allopatric populations (Fig. 11).

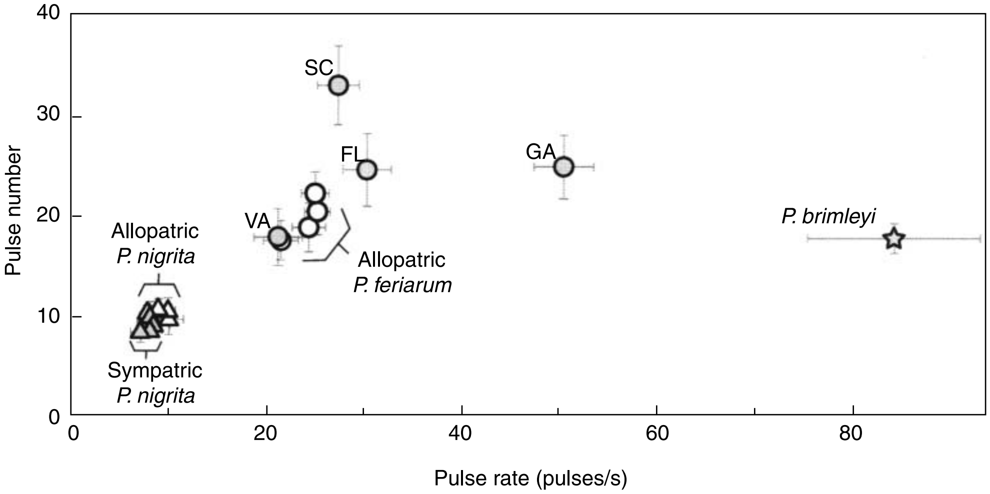


Fig. 7. Acoustic signal variation in *P. feriarum*, *P. nigrita*, and *P. brimleyi* populations with respect to pulse rate and pulse number. Means (symbols) and standard deviations (error bars) are shown. *Pseudacris feriarum* is represented by circles, *P. nigrita* by triangles, and *P. brimleyi* by a star. Shaded and open symbols indicate sympatric and allopatric populations, respectively. Note that the amount of variation among sympatric *P. feriarum* populations is substantially greater than variation among allopatric populations, although geographic distances among populations within each group are similar. In the two populations (FL and GA) in which *P. feriarum* is sympatric with a single heterospecific (*P. nigrita*), the species displaced primarily in pulse rate, and to a lesser degree in pulse number. In the single displaced population (SC) in which *P. feriarum* overlaps with two heterospecifics (*P. nigrita and P. brimleyi*), the calls are displaced only in pulse number. Note that the ancestral (allopatric, open circles) calls of *P. feriarum* were intermediate in pulse rate (x-axis) but not pulse number (y-axis; greater than *P. nigrita*, but equal to *P. brimleyi*) with respect to the two heterospecifics. To avoid signal interference and potential hybridization, this initial state presumably led to displacement of calls of *P. feriarum* only in pulse number where it occurs with the other two taxa. Modified from Lemmon (2009). Reproduced with kind permission from John Wiley and Sons.

Males of *H. cinerea* in syntopic sites also chose more elevated calling positions than did males in allopatric populations.

Höbel and Gerhardt (2003) conducted female-preference tests using synthetic advertisement calls with frogs from four populations of each type. If the amplitude of alternatives was equalized, females from allopatric populations were as selective as females from sympatric areas in playback tests of synthetic calls with spectra typical of conspecific calls versus calls with spectra typical of heterospecific signals. But a female entering a breeding pond must frequently first encounter a heterospecific male, whose calls will of course be more intense at her position. When the relative sound pressure level (SPL) of the conspecific stimulus was reduced, however, nearly 80% of the females from sympatric areas maintained this preference at –12 dB (a factor of 4 – which would correspond to a fourfold difference in source distance if amplitude drop conformed to the inverse distance law). The preference of females from three of the four allopatric areas was abolished at –9 dB (Fig. 12). Females from the fourth allopatric population, which was close to an area of (western) geographic overlap, were as selective as or more so than females from sympatric populations. Finally, in tests with equal-amplitude alternatives, females from all four

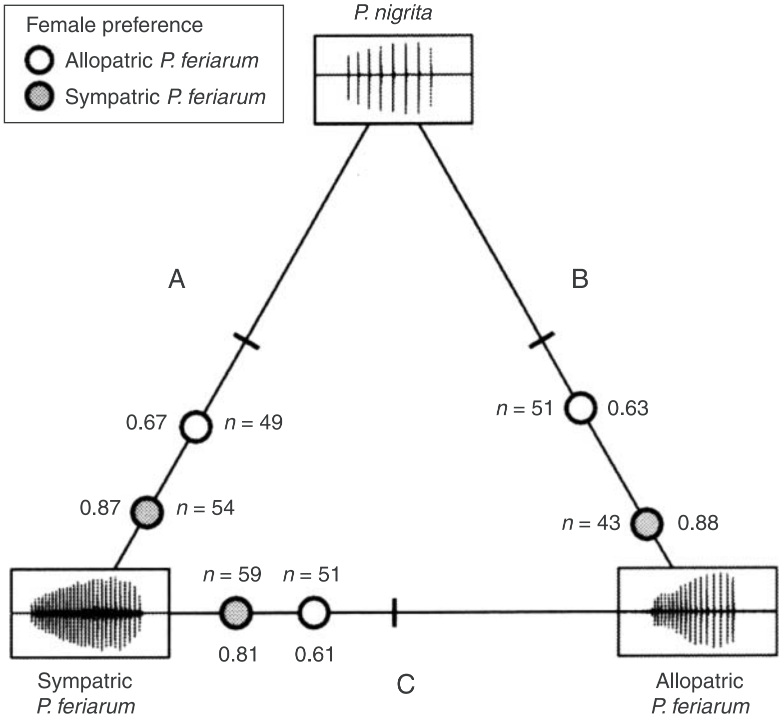


Fig. 8. Summary of female preference results from the three playback experiments (A, B, and C). An oscillogram of a natural call from the respective populations is shown in the box at each vertex of the triangle. The sample size and the proportion of females that chose the more popular stimulus is shown next to each data point. Black tick marks indicate the expectation under no preference. Note that the propensity of females to hybridize has been reduced in sympatry. Also note that sympatric females have the same preference in tests A and B, regardless of the conspecific call presented. This shows that selection has acted on female selectivity, but making the choice more difficult by reducing the relative sound pressure level of the conspecific call may well have revealed the role of reproductive character displacement by showing a weaker preference for the non-displaced call in test B. Modified from Lemmon (2009). Reproduced with kind permission from John Wiley and Sons.

sympatric populations preferred a 'conspecific' synthetic call to an alternative with spectral properties typical of an F1 hybrid; females from only two of the four allopatric populations did so.

My interpretation of these results is that selection has acted mainly to sharpen the acoustic selectivity of females for conspecific calls that already differed sufficiently between the species to allow for recognition. The payoff for this selectivity in turn must have been based mainly on avoiding the production of hybrids with relatively unattractive calls (Gerhardt, 1974a; Höbel and Gerhardt, 2003). Hybrids might also be at an ecological disadvantage because habitats surrounding the usual breeding sites (permanent ponds and lakes in *H. cinerea* vs. temporary or semi-permanent ponds in xeric areas in *H. gratiosa*) of the two species differ significantly (H.C. Gerhardt, personal observation). It would be interesting to learn if the long history of hybridization at the fish hatchery resulted in changes in the genetic make-up and behaviour of individuals of both species in normal habitats in this region. The

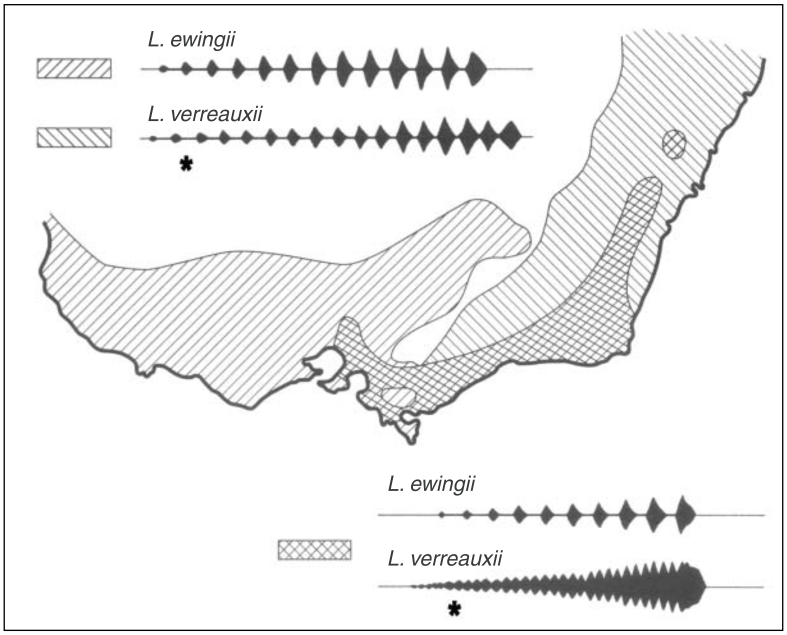


Fig. 9. Map of southeastern Australia showing the distribution of the treefrogs *Litoria ewingii* and *L. verreauxii*. Diagonal hatching shows areas of allopatry and cross-hatching shows area of sympatry. Oscillograms show single notes from advertisement calls. The pulse rate and depth of amplitude modulation between the two species differ not only in sympatric areas, but also between sympatric and allopatric populations of *L. verreauxii* (see asterisks). Both differences are adequate to promote preferences for conspecific calls (*L. ewingii* vs. *L. verreauxii* in sympatry) and for local males (*L. verreauxii*). Modified from Gerhardt and Huber (2002).

geographic variation in preference strength among both sympatric and allopatric populations serves to emphasize again that species interactions (between the two main actors at least) do not explain everything about geographic variation in acoustic communication systems.

The fact that frequency preferences are not intensity-independent, even among sympatric females, almost certainly stems from the lack of a place mechanism in the auditory system of these lower vertebrates, and the same is true of orthopterans (Gerhardt and Huber, 2002). This means that differences in signal frequencies must be much larger than those in birds, for example, whose auditory systems are capable of resolving a 1% difference (Dooling *et al.*, 2000).

Cryptic species of grey treefrogs

In contrast to the green and barking treefrogs, the diploid (*Hyla chrysoscelis*) and biparental tetraploid (*H. versicolor*) grey treefrogs, which are indistinguishable by external morphology, are genetically incompatible. The two species occur in the eastern third of the USA and Canada. There are large regions of allopatry and widespread but scattered areas of sympatry, within which the two species frequently breed synchronously at the same ponds (Figs. 13 and 14). Analyses of advertisement calls of both species are available from dozens of localities, and the selectivity of females of *H. chrysoscelis* has been assessed in 11 populations (Gerhardt, 1995, 1999, unpublished data).

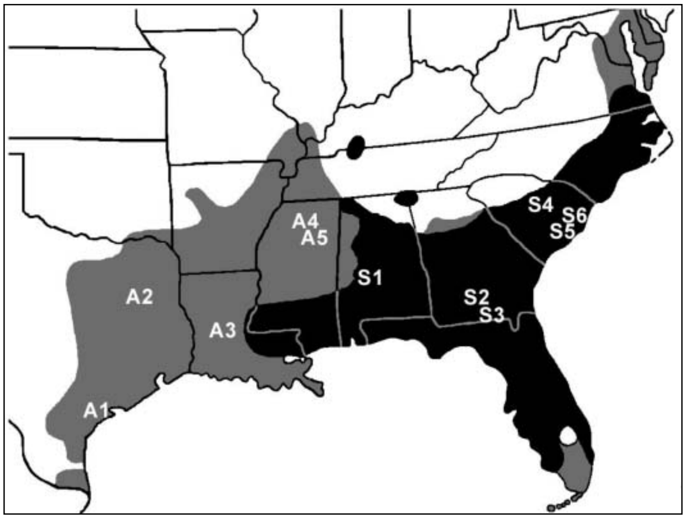


Fig. 10. The locations of the 11 study sites. *Hyla gratiosa* occupies the region with black shading. *Hyla cinerea* lives there as well, as well as in the region with grey shading. The study ponds were situated at: Rob and Bessie Welder Wildlife Refuge (A1), San Patricio Co., Texas; Richland Creek Wildlife Management Area (A2), Freestone Co., Texas; Beechwood State Fish Hatchery (A3), Rapides Co., Louisiana; Private John Allen National Fish Hatchery (A4) and Tombigbee State Park (A5), both Lee Co., Mississippi; Marion State Fish Hatchery (S1), Perry Co., Alabama; Paradise Public Fishing Area (S2), Berrien Co., Georgia; Grand Bay Wildlife Management Area (S3), Loudes Co., Georgia; Southland Fisheries (S4), Calhoun Co., South Carolina; Francis Marion National Forest (S5) and Hobcaw Barony (S6), both Georgetown Co., South Carolina. Sites S2 and S4 were syntopic sites that had both species calling at the time the study was conducted. Ranges based on Conant and Collins (1998) maps. From Höbel and Gerhardt (2003).

The tetraploid species arose multiple times via allopolyploidy, probably involving different ancestral lineages of *H. chrysoscelis* (Holloway *et al.*, 2006). The calls of the three major mtDNA-defined lineages all have the same basic structure: two main spectral peaks with pulse trains having slow rise-time, long-duration pulses. The calls of the three tetraploid lineages are also quantitatively similar. The maximum difference in pulse rate between populations (< 15%) is almost certainly insufficient to mediate reliable assortative mating by lineage (Gerhardt, 2005). Gene flow between tetraploid lineages has also been documented (Espinoza and Noor, 2002; Holloway *et al.*, 2006).

The fine-scale temporal properties (pulse rate and shape) of the advertisement calls of diploids and tetraploids are well differentiated, and the high phonotactic selectivity of females is consistent with the rarity of hybrids (Gerhardt *et al.*, 1994). The selectivity of females of *H. versicolor* for species-typical values of pulse duration, rise-time, and pulse rate results in nearly total discrimination against the calls of *H. chrysoscelis* (Gerhardt, 2005). Discrimination against calls of *H. versicolor* by females of *H. chrysoscelis* is based solely on the difference in pulse rate.

Geographic variation in pulse rate in *H. chrysoscelis* is much more impressive than that in *H. versicolor*. Temperature-corrected differences in this trait between eastern and western populations exceed the 20% level that reliably elicits preferences (Fig. 13), and females from an eastern locality preferred the calls of local males to those of a western population

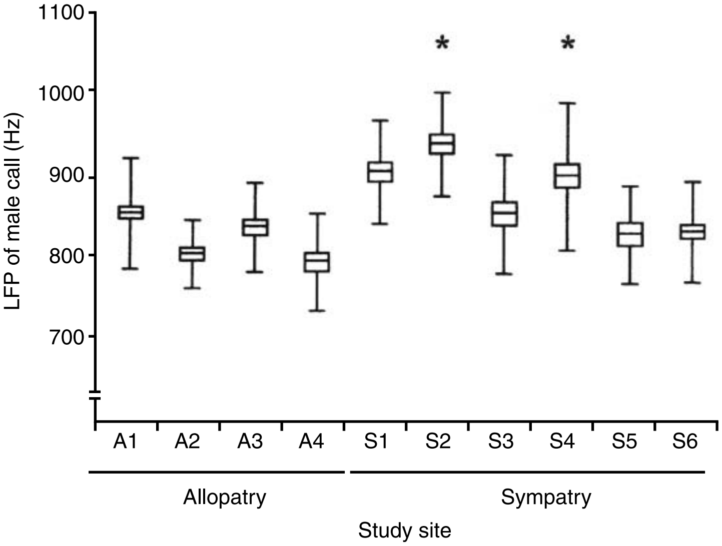


Fig. 11. Dominant frequency of the advertisement calls of male *Hyla cinerea* from four allopatric (A1–A4) and six sympatric (S1–S6) populations. Note the non-clinal nature of the variation in call dominant frequency, and the jump towards higher values at the border from allopatry to sympatry. Although there was no consistent effect of geographic sympatry, males from syntopic ponds (S2 and S4, indicated by asterisks) had higher frequency calls than did males that called from ponds without *H. gratiosa*. Mean values are shown, together with the standard error (box) and the standard deviation (whiskers) for each population. See Fig. 10 for details about the location of the study populations. From Höbel and Gerhardt (2003).

(Gerhardt, 1974b). Even though pulse rate is not the only property that affects mate choice, it is the only trait that differs sufficiently at the population level to distinguish statistically among widely separated populations (Gerhardt, 2012).

The pattern of geographic variation in pulse rate in *H. chrysoscelis* does not, however, conform to the expectations of reproductive character displacement: differences between populations within areas of allopatry are often larger than in any comparison between adjacent sympatric and allopatric populations (e.g. Gerhardt, 1999). Variation in pulse rate is generally clinal, with increasing values from east to west. The cline is more or less smooth across the Gulf coast, but there is a jump from the eastern to the western side of the Appalachian Mountains (Fig. 13). This pattern suggests re-established gene flow in the southern part of the range between formerly isolated groups of populations and retarded gene flow across the mountains.

Shifts in distribution create potential problems for the interpretation of reproductive character displacement in any system. Extensive and scattered areas of allopatry of *H. versicolor* indicate that these frogs expanded their ranges of distribution, displaced *H. chrysoscelis* in some areas, or both. Indeed, there is some evidence of polyploid hybrid (ecological) superiority in the mid-Atlantic area (Otto *et al.*, 2007). Besides the interactions occurring because of sympatric (allopolyploid) speciation, shifts in ranges of distribution have probably also resulted in interactions between tetraploids and 'naïve' *H. chrysoscelis*.

Regardless of these complications, comparisons of the phonotactic selectivity of females of *H. chrysoscelis* from contemporary sympatric and allopatric populations do show a

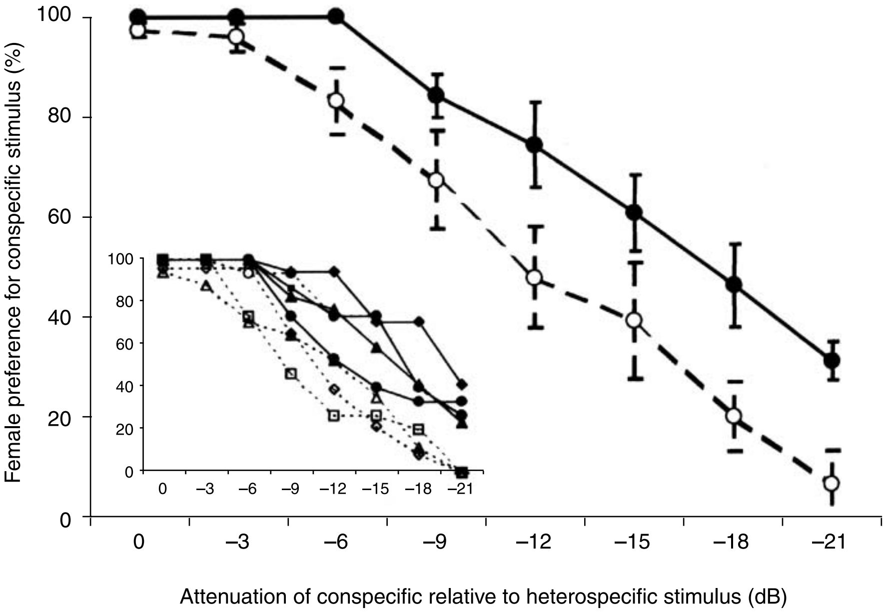


Fig. 12. Preference strength for the conspecific call of females of *Hyla cinerea.* A series of stimulus pairs with increasing amplitude differences favouring the heterospecific call were presented to *H. cinerea* females. Preference strengths of allopatric (open symbols) and sympatric females (filled symbols) were calculated as the mean percentage (± S.E.) of females choosing the conspecific over the heterospecific stimulus in the respective stimulus pairs. The smaller inset shows the same dataset, but individual populations are shown separately (Allopatry: open diamond A1, open square A2, open circle A3, open triangle A4; Sympatry: solid circle S1, solid diamond S2, solid triangle S4, solid square S6). Even though females from Beechwood State Fish Hatchery (A3) showed a preference strength that was comparable or even slightly better than that of females from some sympatric localities, the overall pattern shows greater preference strength in sympatry compared to allopatry. We tested a total of 134 females in this experiment (23 from A1, 15 from A2, 15 from A3, 17 from A4, 15 from S1, 17 from S2, 17 from S6, and 15 from S8). From Höbel and Gerhardt (2003).

strong pattern of reproductive character displacement (Gerhardt, 1995, 1999, unpublished data). As in green and barking treefrogs, call differences between newly formed tetraploids and their diploid parents were apparently sufficient to allow assortative mating, which would have been strongly favoured because of hybrid inviability and infertility. Studies of artificially produced autotriploids of *H. chrysoscelis* show shifts in fine-scale temporal properties in the direction of the tetraploid species that is paralleled by shifts in preference (Keller and Gerhardt, 2001; Tucker and Gerhardt, 2012). These 'pleiotropic' effects of polyploidization, probably acting mainly through cell-size and number changes, thus set the stage for assortative mating and increasing the chances of successful polyploid survival.

In testing for the potential for differences in acoustic signals to promote assortative mating, it is crucial not merely to assess preferences based on average differences. This is especially true when such differences are more than adequate to promote conspecific mate-choice in any population. As in green treefrogs, the demonstration of reproductive character displacement in female selectivity in grey treefrogs depended on testing females

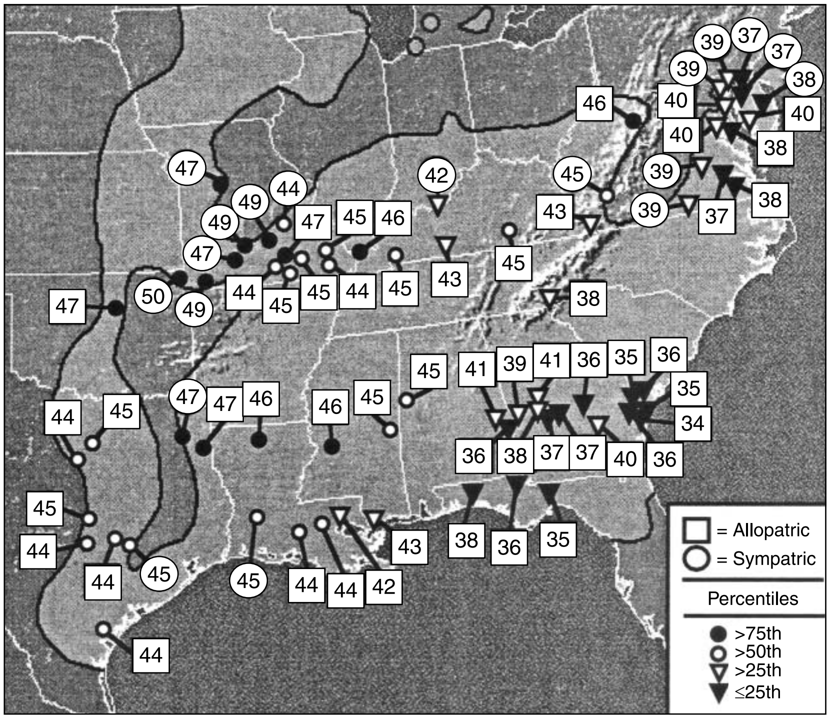


Fig. 13. Variation in pulse rate (pulses per second, corrected to 20°C) in advertisement calls of *Hyla chrysoscelis* across three east–west transects. Numbers in squares = allopatric populations; numbers in circles = sympatric (with *H. versicolor*) populations. There is a relatively smooth east–west cline (increasing pulse rates) in the two southern transects, but note that frogs from allopatric populations on the western side of the Appalachian mountains in the northernmost transect have higher pulse rates than frogs from sympatric populations on the eastern side of the mountains. This pattern is the opposite of that expected of reproductive character displacement in this call property. Figure adapted from unpublished dissertation (University of Missouri) by M. Keller.

with more difficult but realistic choices between signals differing in fine-scale temporal properties. In one set of experiments, the alternatives differed in call duration as well as in pulse rate: the ‘heterospecific’ call was three times longer than the conspecific call. This test pitted the directional preferences for call duration (Bee, 2008), which is a ‘good genes’ indicator (Welch *et al.*, 1998; Welch, 2003), against the stabilizing preferences for pulse rate (Gerhardt, 2005), which indicates genetic compatibility. In a second set of experiments, the alternative calls had the same duration but the amplitude of the ‘conspecific’ alternative was reduced by 12 dB relative to that of the heterospecific alternative. The same rationale was used in estimating the preference strength of sympatric and allopatric females of the green treefrog with regard to spectral call differences (see above). In tests of grey treefrogs, the difference in pulse rate was 30% instead of the usual ≥50% to reflect the fact that temperature mismatches are common within breeding ponds and the difference in pulse rate is reduced when *H. chrysoscelis* calls at cooler temperatures than *H. versicolor*.

Females from all except one sympatric area were highly selective for the alternative with pulse-rate values typical of conspecific males in their own population in both experiments

(Gerhardt, 1994, 1999, unpublished data). In one exceptional population, within 80 km of an extensive area of allopatry, females were unselective in the experiment involving the long-call alternative but highly selective in the experiment with the equal-duration alternative of higher amplitude (C. Gerhardt, unpublished data). Females from a nearby allopatric population (labelled 'CH' in Fig. 13, bottom transect) were unselective in both tests, as were females from most other allopatric populations (Gerhardt, 1999). Females from some allopatric populations in the southeastern USA were, however, nearly as selective as females from syntopic populations in the unequal amplitude tests [75% vs. 80%, respectively, in the unequal amplitude tests (Gerhardt, 1999)]. In central Kentucky, by contrast, only about 27% of the females chose the call with the local pulse rate (Gerhardt, 1999). These results are reminiscent of the geographic variation in preference functions based on spectral properties in *H. cinerea* (Höbel and Gerhardt, 2003), and serve to emphasize again that variation in communication systems occurs within both sympatry and allopatry. A more complete interpretation of these results awaits the completion of phylogeographic research designed to characterize genetic divergence within *H. chrysoscelis* and perhaps to distinguish primary from secondary sympatry.

Tinkerbirds

Perhaps the first convincing case of reproductive character displacement in bird acoustic communication is based on 'interference' selection in tinkerbirds (genus *Pogoniulus*), which are non-passerine species in which songs are presumably unlearned. Kirschel *et al.* (2009a) studied songs, male responses to playbacks, and morphological traits of two species at 27 locations in central Africa (Fig. 14). The range of the yellow-rumped tinkerbird (*P. subsulphureus*) is completely overlapped by that of the yellow-throated tinkerbird (*P. bilineatus*), but areas in which one of the species was very rare were considered to be 'effective allopatry'. This designation is based on the idea that a rare species will have little (interaction) impact on the common one, an assumption supported by the similarities of *P. bilineatus* from true allopatry and effective allopatry (Kirschel *et al.*, 2009a). Indeed, human alterations have fostered contact between the two species, which normally segregate by habitat in areas of geographic sympatry.

The dominant frequency of the song of *P. subsulphureus* was higher in sympatry than in allopatry and there was also a shift to higher song rates (Fig. 14). The dominant frequency in *P. bilineatus* was lower in sympatry than in allopatry, and there was no difference in song rate (Kirschel *et al.*, 2009a). Changes in dominant frequency especially could have been a by-product of the parallel shifts that occurred in body and bill size. Playbacks showed that females and males in sympatric areas were relatively unresponsive to songs of the other species. Male tinkerbirds in allopatry were as likely to respond to heterospecific songs as they were to conspecific ones (Fig. 15).

Kirschel *et al.* (2009a) conclude that reproductive interference is the main driver of sympatric divergence in this system; sympatric males waste less time than allopatric males responding aggressively to songs of the other species. There was no evidence of hybridization, perhaps reflecting the exquisite auditory selectivity of female birds, even in song-learning species in which there is convergence in song properties (Seddon and Tobias, 2010). As discussed below, such selectivity may be one reason for the rarity of reproductive character displacement in birds and mammals: subtle differences arising in allopatry may well be sufficient to promote conspecific mate-choice after re-established contact without

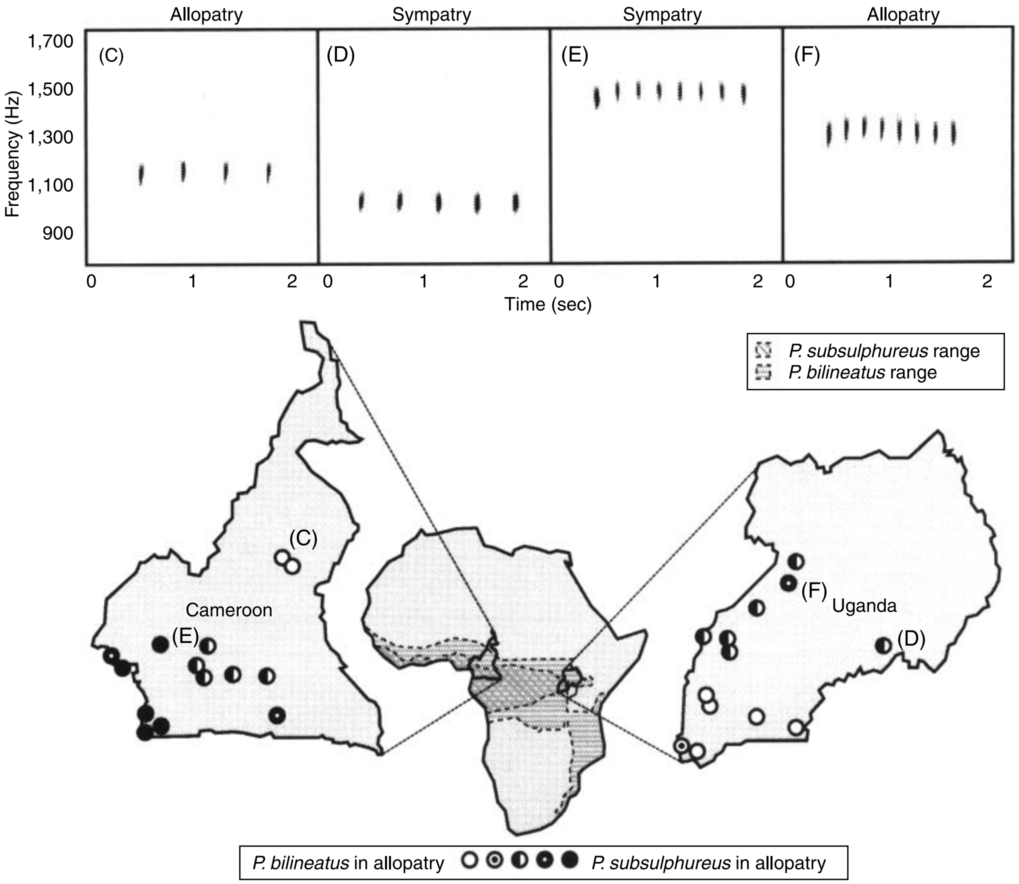


Fig. 14. Songs in sympatry are more different than songs in allopatry. (C–F) Spectrograms are examples of the species' mean song frequencies for allopatric *Pogoniulus bilineatus* at Wakwa (C), sympatric *P. bilineatus* at Mabira Forest (D), sympatric *P. subsulphureus* song at Obala (E), and allopatric *P. subsulphureus* song at Budongo (F). The Africa map illustrates the species' distributions. *Pogoniulus bilineatus* completely encompasses the range of *P. subsulphureus*, but in lowland rainforests *P. bilineatus* is rare. Site locations are illustrated for *P. bilineatus* in allopatry (open circles); *P. subsulphureus* in allopatry (solid circles); *P. subsulphureus* common, *P. bilineatus* rare, and thus effective allopatry for *P. subsulphureus* but sympatry for *P. bilineatus* (solid circles with white dots); *P. bilineatus* common, *P. subsulphureus* rare, and thus effective allopatry for *P. bilineatus* (open circles with black dots); and both species common in sympatry (half-filled circles). Adapted from Kirschel *et al.* (2009a). Reproduced with kind permission from PNAS.

additional divergence in sympatry. Furthermore, I suggest that interference selection that acts against males in a territorial context as suggested for tinkerbirds and other species (e.g. Grether *et al.*, 2009) is probably much less potent than selection against mating mistakes by females.

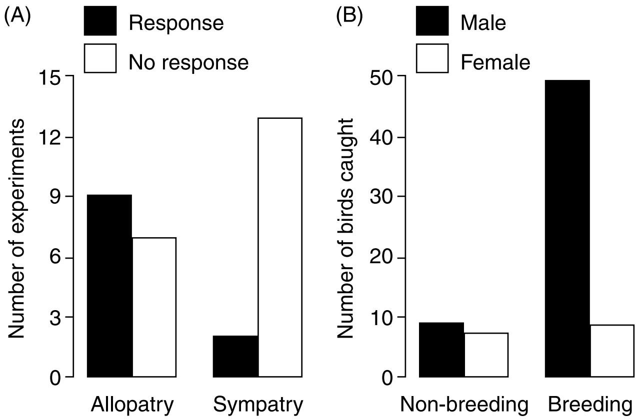


Fig. 15. Allopatric tinkerbirds respond significantly more than sympatric tinkerbirds to heterospecific playback. (A) Bar charts indicate responses to playback of heterospecific song to both species. (B) Males respond significantly more than females to conspecific playback when they are in breeding condition, whereas there is no difference in responses between the sexes when they are not breeding. From Kirschel *et al.* (2009a). Reproduced with kind permission from PNAS.

DISCUSSION

Reproductive character displacement: multiple causes and effects

Synoptic assemblages of closely related species have distinctive acoustic communication signals and seldom hybridize because of mistakes in signal identity. Theoretical and empirical studies indicate that reproductive character displacement may sometimes be an intermediate path to this state of affairs, but non-interactive attainment of sympatry is likely to be much more prevalent. In the examples summarized above, the primary changes in acoustic communication systems, which were the primary mediators of mate choice and male–male competition, presumably resulted in increased reproductive fitness by reducing costly mistakes in signal identification. On the one hand, these positive consequences in some systems occurred because, independent of renewed contact (or at the time of polyploid speciation in grey treefrogs), the taxa had diverged sufficiently so that hybrids were genetically incompatible (field crickets, chorus and grey treefrogs, and perhaps periodical cicadas) or behaviourally and perhaps ecologically unfit (green and barking treefrogs). On the other hand, shifts in the signals of periodical cicadas and tinkerbirds increased reproductive success by reducing masking interference or misidentification of territorial rivals, even if hybrids are not genetically incompatible or otherwise unfit (periodical cicadas?) or rare (tinkerbirds, which could use visual cues at close range to prevent mismating).

Why are robust examples rare: theoretical considerations

Reproductive character displacement is expected to be uncommon to rare for several theoretical reasons. First, there must be sufficient ecological divergence in allopatry to allow reasonably broad overlap in distributions and breeding times and places when contact is re-established. Second, there are Goldilocks requirements for the initial magnitude of signal differences. Differences in mate-attracting signals must be sufficient to distinguish at least some individuals of the interacting taxa yet not so well diverged that mating mistakes are rare. Indeed, as reproductive character displacement proceeds and hybridization decreases, the pattern itself could be obscured (e.g. Alexander *et al.*, 1997). Finally, the genetic architecture underlying the communication system may determine whether speciation is realized (Bank *et al.*, 2012). Third, there must be optimal ranges of gene flow into and out of the area of overlap relative to the magnitude of selection against hybrids. Recent empirical (Nosil and Crespi, 2004) and experimental (Matute, 2010) studies are significant because they provide evidence that reproductive character displacement can occur despite the 'gene flow' problem.

Robust empirical demonstrations of reproductive character displacement are scarce because of practical difficulties that include: (1) the high likelihood that present-day distributions of interacting species have shifted from distributions at the time of their initial contact (e.g. Canestrelli *et al.*, 2007); (2) uncertainty about the magnitude and causes of hybrid unfitness and even the taxonomic status of the interacting entities (e.g. Higgins and Waugaman, 2004); (3) uncertainty about the behavioural effectiveness of differences in acoustic signals; and (4) uncertainty about the direction, magnitude, and impact of gene flow.

Despite the advantages of studying acoustic communication, my criteria for choosing robust examples eliminated a handful of studies where sampling was inadequate or no information was available about the efficacy of signal differences to promote assortative mating (for a review, see Gerhardt and Huber, 2002). These deficiencies are not surprising. Whereas recording sufficient samples of acoustic signals from multiple populations is difficult enough, obtaining reliable responses in well-designed playback experiments from multiple populations is especially onerous. The payoff, however, is that by accumulating data from studies that robustly demonstrate reproductive character displacement or the lack thereof, we can ultimately estimate the frequency of this phenomenon and hence its role in promoting speciation or maintaining genetic divergence between taxa that re-establish contact after long periods of isolation.

Taxonomic differences in causes of geographic variation on communication

In many kinds of insects and frogs, the acoustic modality dominates mate-choice preferences, especially in nocturnally breeding species. Insects are somewhat more likely than frogs to use olfactory cues as secondary mate-choice criteria (Gerhardt and Huber, 2002). Most birds, in which long-range acoustic signals are common, can rely on visual cues at close range (e.g. Gonzalez-Voyer *et al.*, 2013), although there may be exceptions among non-song-learning species in light-limited environments (Seddon, 2005). Long-range signals are much less common in mammals, which can also base mate-choice on visual and olfactory differences at close range. Campbell *et al.* (2010) noted the lack of any convincing example of reproductive character displacement in acoustic signals in mammals; they also showed that genetic drift is the most likely explanation for geographic variation in the 'long-range' advertisement signals of singing mice.

Even if acoustic signals are prominent in mate choice in a bird or mammal, their more sophisticated auditory systems allow individuals to discriminate more subtle differences in acoustic signals than insects and lower vertebrates, which, for example, lack the ability to discriminate small differences in frequency independent of intensity (Gerhardt and Huber, 2002). The greater auditory resolution, multi-modal communication, and more sophisticated social systems make it likely that mating mistakes based on misidentification of acoustic signals is very rare in higher vertebrates (Seddon and Tobias, 2010). Even if reproductive character displacement occurs between genetically incompatible species, it will probably involve selection on receivers rather than signals and hence go undetected by researchers. Finally, learning can have unexpected consequences on geographic variation and speciation, especially because genetic incompatibility seems to evolve much more slowly than morphological and behavioural traits (Grant and Grant, 1997). Long-term studies of communities of Darwin's finches, for example, show that mistakes in song learning can even contribute to acoustic signal divergence that results in speciation by hybridization (e.g. Grant and Grant, 2010).

When communicating at long range, masking interference and the effects of the environment on signal transmission is likely to have more of an influence on birds than on insects and frogs. One reason is that masking interference by conspecifics in their typically dense breeding choruses is likely to be more potent than interspecific masking (e.g. Gerhardt and Klump, 1988). Most attraction to calling individuals also takes place at relatively close range, so that environmental effects on signals probably have little effect on orientation to the breeding pond (Christie *et al.*, 2010; Kuczynski *et al.*, 2010) if the species in question even uses such cues (Murphy, 2003; Wells, 2007). Two comparative studies of environmental effects on frog-call transmission found that heterospecific signals often suffered the same or even less degradation than conspecific signals in an animal's normal habitat (e.g. Penna and Solís, 1998; Kime *et al.*, 2000). Ryan and Wilczynski (1991) documented geographic variation in advertisement calls in the cricket frog (*Acris crepitans*) across a long east–west transect in Texas but detected only weak effects of habitat acoustics. By contrast, habitat effects and masking (even by human sources) are well documented in studies of acoustic communication in birds (Kirschel *et al.*, 2009a, 2009b; Tobias *et al.*, 2010). Although these effects can influence geographic variation in acoustic signals, they are unlikely to drive reproductive character displacement because mistakes in interspecific territoriality are almost certainly less costly than mating mistakes leading to less fit hybrids.

In summary, I conclude that there are many paths to reproductive character displacement and multiple factors that interact with sexual selection to generate geographical variation in both areas of sympatry and allopatry. Better resolution of patterns and processes requires measuring phenotypic traits, including molecular markers, in numerous populations and assessing their biological consequences on mate choice. Studies of acoustic communication systems offer many advantages in executing these difficult tasks.

REFERENCES

Alexander, R.D., Marshall, D.C. and Cooley, J.R. 1997. Evolutionary perspectives on insect mating. In *The Evolution of Mating Systems in Insects and Arachnids* (J.C. Choe and B.J. Crespi, eds.), pp. 4–31. Cambridge: Cambridge University Press.

Amézquita, A., Hödl, W., Lima, A.P., Castellanos, L., Erdtmann, L. and De Araújo, M.C. 2006. Masking interference and the evolution of the acoustic communication system of the Amazonian dendrobatid frog *Allobates femoralis*. *Evolution*, **60**: 1874–1887.

Bank, C., Hermisson, J. and Kirkpatrick, M. 2012. Can reinforcement complete speciation? *Evolution*, **66**: 229–239.

Bee, M.A. 2008. Parallel preferences for call duration in a diploid ancestor of an allotetraploid treefrog. *Anim. Behav.*, **76**: 845–853.

Butlin, R. 1989. Reinforcement of premating isolation. In *Speciation and its Consequences* (D. Otte and J.A. Endler, eds.), pp. 158–179. Sunderland, MA: Sinauer Associates.

Campbell, P., Pasch, B., Pino, J.L., Crino, O.L., Phillips, M. and Phelps, S. M. 2010. Geographic variation in the songs of neotropical singing mice: testing the relative importance of drift and local adaptation. *Evolution*, **64**: 1955–1972.

Canestrelli, D., Cimmaruta, R. and Nascetti, G. 2007. Phylogeography and historical demography of the Italian treefrog *Hyla intermedia* reveals multiple refugia, range expansions and secondary contacts within the Italian peninsula. *Mol. Ecol.*, **16**: 4808–4821.

Castellano, S. and Rosso, A. 2007. Female preferences for multiple attributes in the acoustic signals of the Italian treefrog, *Hyla intermedia*. *Behav. Ecol. Sociobiol.*, **61**: 1293–1302.

Christie, K., Schul, J. and Feng, A.S. 2010. Phonotaxis to male's calls embedded within a chorus by female gray treefrogs, *Hyla versicolor*. *J. Comp. Physiol. A*, **196**: 569–570.

Conant, R. and Collins, J.T. 1998. *A Field Guide to Reptiles and Amphibians: Eastern and Central North America*. Boston, MA: Houghton-Mifflin.

Cooley, J.R., Simon, C., Marshall, D.C., Slon, K. and Ehrhardt, C. 2001. Allochronic speciation, secondary contact, and reproductive character displacement in periodical cicadas (Hemiptera: *Magicicada* spp.): genetic, morphological, and behavioural evidence. *Mol. Ecol.*, **10**: 661–671.

Cooley, J.R., Marshall, D.C., Hill, K.B.R. and Simon, C. 2006. Reconstructing asymmetrical reproductive character displacement in a periodical cicada contact zone. *J. Evol. Biol.*, **19**: 855–868.

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

Doherty, J.A. and Callos, J.D. 1991. Acoustic communication in the trilling field cricket, *Gryllus rubens* (Orthoptera: Gryllidae). *J. Insect Behav.*, **4**: 67–81.

Dooling, R.J., Lohr, B. and Dent, M.L. 2000. Hearing in birds and reptiles. In *Comparative Hearing: Birds and Reptiles* (D.J. Dooling, A.N. Popper and R.R. Fay, eds.), pp. 308–359. New York: Springer Verlag.

Espinoza, N.R. and Noor, M.A. 2002. Population genetics of a polyploid: is there hybridization between lineages of *Hyla versicolor? J. Hered.*, **93**: 81–85.

Fouquette, M.J. 1975. Speciation in chorus frogs. I. Reproductive character displacement in the *Pseudacris nigrita* complex. *Syst. Zool.*, **24**: 16–22.

Gabor, C.R. and Ryan, M.J. 2001. Geographical variation in reproductive character displacement in mate choice by male sailfin mollies. *Proc. R. Soc. Lond. B*, **268**: 1063–1070.

Gerhardt, H.C. 1974a. Vocalizations of some hybrid treefrogs: acoustic and behavioural analyses. *Behaviour*, **49**: 130–151.

Gerhardt, H.C. 1974b. Mating call differences between eastern and western populations of the treefrog *Hyla chrysoscelis*. *Copeia*, **1974**: 543–546.

Gerhardt, H.C. 1994. Reproductive character displacement on female mate choice in the grey treefrog *Hyla chrysocelis*. *Anim. Behav.*, **47**: 959–969.

Gerhardt, H.C. 1995. Phonotaxis in female frogs and toads: execution and design of experiments. In *Animal Psychophysics: Design and Conduct of Sensory Experiments* (G.M. Klump, R.R. Dooling, R.R. Fay and W.C. Stebbins, eds.), pp. 209–220. Basel: Birkhäuser Verlag.

Gerhardt, H.C. 1999. Reproductive character displacement and other sources of environmental selection on acoustic communication systems. In *The Design of Animal Communication* (M. Hauser and M. Konishi, eds.), pp. 515–534. Cambridge: MIT Press.

Gerhardt, H.C. 2005. Advertisement-call preferences in diploid-tetraploid treefrogs (*Hyla chrysoscelis* and *Hyla versicolor*): implications for mate choice and the evolution of communication systems. *Evolution*, **59**: 395–408.

Gerhardt, H.C. 2012. Evolution of acoustic communication: a multi-level analysis of signal variation. *Bioacoustics*, **21**: 9–11.

Gerhardt, H.C. and Brooks, R. 2009. Experimental analysis of multivariate female choice in gray treefrogs (*Hyla versicolor*): evidence for directional and stabilizing selection. *Evolution*, **63**: 2504–2512.

Gerhardt, H.C. and Davis, M.S. 1988. Variation in the coding of species identity in the advertisement calls of *Litoria verreauxii* (Anura: Hylidae). *Evolution*, **42**: 556–565.

Gerhardt, H.C. and Huber, F. 2002. *Acoustic Communication in Insects and Anurans*. Chicago, IL: University of Chicago Press.

Gerhardt, H.C. and Klump, G.M. 1988. Masking of acoustic signals by the chorus background noise in the green tree frog: a limitation on mate choice. *Anim. Behav.*, **36**: 1247–1249.

Gerdhardt, H.C., Guttman, S.I. and Karlin, A. 1980. Natural hybrids between *Hyla cinerea* and *Hyla gratiosa*: morphology, vocalization and electrophoretic analysis. *Copeia*, **1980**: 577–584.

Gerhardt, H.C., Ptacek, M.B., Barnett, L. and Torke, K.G. 1994. Hybridization in the diploid-tetraploid tree frogs *Hyla chrysoscelis* and *Hyla versicolor*. *Copeia*, **1994**: 51–59.

Goldberg, E.E. and Lande, R. 2006. Ecological and reproductive character displacement on an environmental gradient. *Evolution*, **60**: 1344–1357.

Gonzalez-Voyer, A., Den Tex, R.-J., Castelló, A. and Leonard, J.A. 2013. Evolution of acoustic and visual signals in Asian barbets. *J. Evol. Biol.*, **26**: 647–659.

Grant, B.R. and Grant, P.R. 2010. Songs of Darwin's finches diverge when a new species enters the community. *Proc. Natl. Acad. Sci. USA*, **107**: 20156–20163.

Grant, P.R. and Grant, B.R. 1997. Genetics and the origin of bird species. *Proc. Natl. Acad. Sci. USA*, **94**: 7768–7775.

Grether, G.F., Losin, N., Anderson, C.N. and Okamoto, K. 2009. The role of interspecific interference competition in character displacement and the evolution of competitor recognition. *Biol. Rev.*, **84**: 617–635.

Higgins, L.A. and Waugaman, R.D. 2004. Sexual selection and variation: a multivariate approach to calls and preferences. *Anim. Behav.*, **68**: 1139–1153.

Hill, K.G., Loftus-Hills, J.J. and Gartside, D.F. 1972. Premating isolation between the Australian field crickets *Teleogryllus commodus* and *T. oceanicus* (Orthoptera: Gryllidae). *Aust. J. Zool.*, **20**: 153–163.

Höbel, G. and Gerhardt, H.C. 2003. Reproductive character displacement in the acoustic system of green treefrogs (*Hyla cinerea*). *Evolution*, **57**: 894–904.

Holloway, A.K., Cannatella, D.C., Gerhardt, H.C. and Hillis, D.M., 2006. Polyploids with different origins and ancestors form a single sexual polyploidy species. *Am. Nat.*, **167**: E88–E101.

Hoskin, C.J. and Higgie, M. 2010. Speciation via species interactions: the divergence of mating traits within species. *Ecol. Lett.*, **13**: 409–420.

Hoskin, C.J., Higgie, M., McDonald, K.R. and Moritz, C. 2005. Reinforcement drives rapid allopatric speciation. *Nature*, **437**: 1353–1356.

Howard, D.J. 1993. Reinforcement: origin, dynamics, and fate of an evolutionary hypothesis. In *Hybrid Zones and the Evolutionary Process* (R.G. Harrison, ed.), pp. 46–69. New York: Oxford University Press.

Jang, Y. and Gerhardt, H.C. 2006a. Divergence in the calling songs between sympatric and allopatric populations of the southern wood cricket *Gryllus fultoni* (Orthoptera: Gryllidae). *J. Evol. Biol.*, **19**: 459–472.

Jang, Y. and Gerhardt, H.C. 2006b. Divergence in female calling song discrimination between sympatric and allopatric populations of the southern wood cricket *Gryllus fultoni* (Orthoptera: Gryllidae). *Behav. Ecol. Sociobiol.*, **60**: 150–158.

Jang, Y., Bockhorst, A. and Gerhardt, H.C. 2007. Reproductive isolation in the wood cricket *Gryllus vernalis* (Orthoptera: Gryllidae). *Ethology*, **113**: 87–96.

Keller, M.D. and Gerhardt, H.C. 2001. Polyploidy affects call structure in gray treefrogs. *Proc. R. Soc. Lond. B*, **268**: 341–345.

Kime, N.M., Turner, W.R. and Ryan, M.J. 2000. The transmission of advertisement calls in Central American frogs. *Behav Ecol.*, **11**: 71–83.

Kirschel, A.N.G., Blumstein, D.T., Cohen, R.E., Buermann, W., Smith, T.B. and Slabbekoorn, H. 2009a. Birdsong tuned to the environment: green hylia song varies with elevation, tree cover and noise. *Behav. Ecol.*, **20**: 1089–1095.

Kirschel, A.N.G., Blumstein, D.T. and Smith, T.B. 2009b Character displacement of song and morphology in African tinkerbirds. *Proc. Natl. Acad. Sci. USA*, **106**: 8256–8261.

Kuczynski, M.C., Vélez, A., Schwartz, J.J. and Bee, M.A. 2010. Sound transmission and the recognition of temporally degraded sexual advertisement signals in Cope's gray treefrog (*Hyla chrysoscelis*). *J. Exp. Biol.*, **213**: 2840–2850.

Lamb, T. and Avise, J.C. 1985. Directional introgression of mitochondrial DNA in a hybrid population of tree frogs: the influence of mating behavior. *Proc. Natl. Acad. Sci USA*, **83**: 2526–2530.

Lemmon, E.M. 2009. Divergence of conspecific signals in sympatry: geographic overlap drives multidimensional reproductive character displacement in frogs. *Evolution*, **63**: 1155–1170.

Lemmon, E.M. and Lemmon, A.R. 2010. Reinforcement in chorus frogs: lifetime fitness estimates including intrinsic natural selection and sexual selection against hybrids. *Evolution*, **64**: 1748–1761.

Liou, L.W. and Price, T.D. 1994. Speciation by reinforcement of premating isolation. *Evolution*, **48**: 1451–1459.

Littlejohn, M.J. 1965. Premating isolation in the *Hyla ewingi* complex (Anura: Hylidae). *Evolution*, **19**: 234–243.

Littlejohn, M.J. 1993. Homogamy and speciation: a reappraisal. In *Oxford Surveys of Evolutionary Biology*, Vol. 9 (D. Futuyma and J. Antonovics, eds.), pp. 135–164. Oxford: Oxford University Press.

Littlejohn, M.J. and Loftus-Hills, J.J. 1968. An experimental evaluation of premating isolation in the *Hyla ewingi* complex (Anura: Hylidae). *Evolution*, **22**: 659–663.

Loftus-Hills, J.J. and Littlejohn, M.J. 1971. Pulse repetition rate as the basis for mating call discrimination by two sympatric species of *Hyla. Copeia*, **1971**: 154–156.

Loftus-Hills, J.J. and Littlejohn, M.J. 1992. Reinforcement and reproductive character displacement in *Gastrophryne carolinensis* and *G. olivacea* (Anura: Microhylidae): a reexamination. *Evolution*, **46**: 896–906.

Marshall, D.C. and Cooley, J.R. 2000. Reproductive character displacement and speciation in periodical cicadas, with description of a new species, 13-year *Magicicada neotredecim*. *Evolution*, **54**: 1313–1325.

Matute, D.R. 2010. Reinforcement can overcome gene flow during speciation in *Drosophila*. *Curr. Biol.*, **20**: 2229–2233.

Mendelson, T.C. and Shaw, K.L. 2012. The (mis)concept of species recognition. *Trends Ecol. Evol.*, **27**: 421–427.

Murphy, C.G. 2003. The cause of correlations between nightly numbers of male and female barking treefrogs (*Hyla gratiosa*) attending choruses. *Behav. Ecol.*, **14**: 274–281.

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

Oldham, R.S. and Gerhardt, H.C. 1975. Behavioral isolation of the treefrogs *Hyla cinerea* and *Hyla gratiosa*. *Copeia*, **1975**: 223–231.

Oh, K.P., Fergus, D.J., Grace, J.L. and Shaw, K.L. 2012. Interspecific genetics of speciation phenotypes: song and preference coevolution in Hawaiian crickets. *J. Evol. Biol.*, **25**: 1500–1512.

Otte, D. 1989. Speciation in Hawaiian crickets. In *Speciation and its Consequences* (D. Otte and J.A. Endler, eds.), pp. 482–526. Sunderland, MA: Sinauer Associates.

Otto, C.R.V., Snodgrass, J.W., Forester, D.C., Mitchell, J.C. and Miller, R.W. 2007. Climatic variation and the distribution of an amphibian polyploid complex. *J. Anim. Ecol.*, **76**: 1053–1061.

Penna, M. and Solís, R. 1998. Frog call intensities and sound propagation in the South American temperate forest region. *Behav. Ecol. Sociobiol.*, **42**: 371–381.

Perrill, S.A., Gerhardt, H.C. and Daniel, R. 1978. Sexual parasitism in the green treefrog (*Hyla cinerea*). *Science*, **200**: 1179–1180.

Pfennig, K.S. and Pfennig, D.W. 2009. Character displacement: ecological and reproductive responses to a common evolutionary problem. *Quart. Rev. Biol.*, **84**: 253–276.

Ryan, M.J. and Rand, A.S. 2003. Sexual selection and female preference space: how female túngara frogs perceive and respond to complex population variation in acoustic mating signals. *Evolution*, **57**: 2608–2618.

Ryan, M.J. and Wilczynski, W. 1991. Evolution of intraspecific variation in the advertisement call of a cricket frog (*Acris crepitans*, Hylidae). *Biol. J. Linn. Soc.*, **44**: 249–271.

Schlefer, E.K., Romano, M.A., Guttman, S.I. and Ruth, S.B. 1986. Effects of twenty years of hybridization in a disturbed habitat on *Hyla cinerea* and *Hyla gratiosa*. *J. Herpetol.*, **20**: 210–221.

Seddon, N. 2005. Ecological adaptation and species recognition drives vocal evolution in neotropical suboscine birds. *Evolution*, **39**: 200–215.

Seddon, N. and Tobias, J.A. 2010. Character displacement from the receiver's perspective: species and mate recognition despite convergent signals in suboscine birds. *Proc. R. Soc. Lond. B*, **277**: 2475–2483.

Servedio, M.R. and Kirkpatrick, M. 1997. 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.

Shaw, K.L. 1996. Sequential radiations and patterns of speciation in the Hawaiian cricket genus *Laupala*, inferred from DNA sequences. *Evolution*, **50**: 237–255.

Smith, M.J., Osborne, W., Hunter, D. and Douglas, M.E. 2003. Geographic variation in the advertisement call structure of *Litoria verreauxii* (Anura: Hylidae). *Copeia*, **2003**: 750–758.

Tobias, J.A., Aben, J., Brumfield, R.T., Derryberry, E.P., Halfwerk, W., Slabbekoorn, H. *et al.* 2010. Song divergence by sensory drive in Amazonian birds. *Evolution*, **64**: 2820–2839.

Tucker, M.A. and Gerhardt, H.C. 2012. Parallel changes in mate-attracting calls and female preferences in autotriploid tree frogs. *Proc. R. Soc. Lond. B*, **279**: 1583–1587.

Veech, J.A., Benedix, J.H. and Howard, D.J. 1996. Lack of calling song displacement between two closely related ground crickets. *Evolution*, **50**: 1982–1989.

Welch, A.M. 2003. Genetic benefits of a female mating preference in gray treefrogs are context-dependent. *Evolution*, **57**: 883–893.

Welch, A.M., Semlitsch, R.L. and Gerhardt, H.C. 1998. Call duration as an indicator of genetic quality in male gray treefrogs. *Science*, **280**: 1928–1930.

Wells, K.D. 2007. *The Ecology and Behavior of Amphibians*. Chicago, IL: University of Chicago Press.