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THE ROLE OF ENVIRONMENTAL SELECTION IN INTRASPECIFIC DIVERGENCE OF MATE RECOGNITION SIGNALS IN THE CRICKET FROG, *ACRIS CREPITANS*

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Divergence of mate recognition signals between closely related taxa is a crucial component in the process of speciation because it can give rise to ethological isolation. The process by which mate recognition signals diverge has been a controversial issue in evolutionary biology. Dobzhansky's (1937) hypothesis of reinforcement suggested that such signals might begin to diverge in allopatry, but to become efficient isolating barriers there must be further selection on the signals during secondary contact between the two taxa. Others (e.g., Mayr, 1942; Lande, 1981; Paterson, 1982; West Eberhard, 1983) have suggested that divergence of mate recognition signals is an incidental by-product of speciation; the species isolating effect is not an evolved function but an incidental consequence. Divergence can result from several causes: stochastic factors, such as genetic drift (Lande, 1981); indirect selection, such as a correlated response to selection on other traits (e.g., body size; Nevo and Capranica, 1985); or direct selection on the signal itself, if sexual selection favored different aspects of the signal in different populations (West Eberhard, 1982) or if the environment favored different call types in different habitats for enhanced transmission efficiency (Morton, 1975). The purpose of this study is to examine the role of environmental selection in the divergence of mate recognition signals between the two subspecies of the cricket frog, *Acris crepitans* (Hylidae).

Cricket frogs are common throughout much of North America east of the Rocky Mountains. Males produce groups of short, click-like advertisement calls that attract females and repel males.

Acris crepitans consists of two subspecies that differ in morphology (Nevo, 1973), allozymes (Dessauer and Nevo, 1969; Salthe and Nevo, 1969), advertisement calls (Nevo and Capranica, 1985), and habitat (Nevo, 1973). In Texas, which includes the zone of parapatry for the two subspecies, *A. c. crepitans* is restricted to the pinewoods of the east, while *A. c. blanchardi* inhabits more open grasslands and plains in the west.

Nevo and Capranica (1985; see also Capranica et al., 1973) examined geographic variation in the advertisement call of *A. crepitans* across its range in North America, and Ryan and Wilczynski (1988, in press) conducted a more fine-scale analysis of call variation across Texas, concentrating on the area of parapatry of the two subspecies. These studies reveal similar patterns. First, there are statistically significant differences in most call characters between subspecies. In general, calls of *A. c. crepitans* have a shorter duration, fewer pulses, a higher dominant frequency, are repeated at a faster rate within the call group, and contain more calls per call group than calls of *A. c. blanchardi*. Second, both studies have shown that these differences in the calls can result in local mate preferences. Although both of these phenomena (call difference and local mate preferences) also can exist within a subspecies, they are more pronounced between subspecies.

Nevo and Capranica (1985) suggested three hypotheses for the evolution of call differences between *A. c. crepitans* and *A. c. blanchardi*: (1) reproductive character displacement between the two subspecies, which they suggest might be incipient ecospecies; (2) a response to indirect selection for body size, which increases from east to west due to selection for the larger *A. c. blanchardi* to avoid desiccation in drier habitats (Nevo, 1973); (3) a response to direct selection on the call due to environmental selection for increased transmission efficiency. The detailed analysis of geographic variation across the zone of parapatry by Ryan and Wilczynski (in press) rejected the first two hypothesis. In this study we test the hypothesis that environmental selection on the call is responsible for the divergence of mate recognition signals between *A. c. crepitans* and *A. c. blanchardi*.

Nevo and Capranica's third hypothesis is based on well documented environmental effects on sound transmission, and on the structural differences between the habitats in which these two subspecies occur. Since Morton's (1975) pioneering study, many other studies have shown that acoustic signals exhibit different rates of attenuation (Marten and Marler, 1977; Marten et al., 1977; Waser and Waser, 1977; Bowman, 1983; Wilczynski et al., 1989) and degradation (Richards and Wiley, 1978, 1982; Gish and Morton, 1981; Ryan and Sullivan, 1989) in different habitats. Several studies examining variation within a species or a group of closely related species also have shown adaptation of bird song structure to local habitat (Hunter and Krebs, 1979; Gish and Morton, 1981; Bowman, 1983).

MATERIALS AND METHODS

We selected calls of three individuals each from a population of *A. c. crepitans* in east Texas (Polk County) pine forest and a population of *A. c. blanchardi* in the open habitat of central Texas (Travis County). Calls were broadcast at ca. 100 dB SPL (re. 20 μPa) at 1 m from the source, the natural calling intensity of a male cricket frog, using a Sony TCD 5M tape recorder and ADS L200 C speaker. Calls were recorded simultaneously with two Marantz PMD 420 stereo tape recorders and four Sennheiser ME80 microphones with

K3U power modules at the following distances: 1 m, 4 m, 8 m, 16 m. Estimates of call attenuation and neural thresholds have predicted the distance over which a female could detect a call to be 18 m (Fox, 1988). All experiments were conducted in early evening, the time when cricket frogs usually begin their calling. These experiments were conducted in two habitats. The open habitat was a large expanse of grassland in the Post-Oak Savanna biome of central Texas (Travis County) and the forest habitat was in the Pineywoods biome of Bastrop County, also in central Texas. This forest, referred to as the "lost pines," is the same biome that characterizes eastern Texas. The experiments were conducted in two sites within each habitat.

Degradation is the amount of change in temporal and spectral aspects of a signal. We used a cross correlation analysis to quantify degradation as a function of distance, similar to the method used by Brown and Waser (1988) to quantify primate call degradation. The higher the cross correlation coefficient, the more similar are two calls, and thus the less degradation. The coefficients were determined with a DATA 6000 digital waveform analyzer, and calls were digitized at a rate of 20 kHz. The call recorded at 1 m was used as the template. We determined the cross correlation coefficients between the call at 1 m and the identical call that was recorded simultaneously at 4 m, 8 m, and 16 m. We correlated the Fourier spectra (frequency versus energy) instead of the oscillograms. In the Fourier spectra the points are standardized along the frequency axis. In the oscillograms, the points often are slightly shifted in different calls depending on exactly when sampling began, thus yielding artificially low correlation coefficients. Correlations between Fourier spectra are independent of differences in amplitude between the two signals. The Fourier spectrum contains all the essential temporal and spectral information in the oscillogram, with the exception of phase; however, there is no evidence that anurans can process phase information (Zakon and Wilczynski, 1988).

We analyzed three calls from each of the six call groups broadcast, one from the beginning, middle, and end of the call group. The identical calls were used in all analyses. Thus for each transect we analyzed nine calls per subspecies at each of the three distances. Since transects were replicated within habitats for each habitat we analyzed 18 calls per subspecies at each distance.

Cross correlation coefficients vary between 0 and a maximum of 1, and thus were transformed for analysis by taking the square root of the arcsine. Data were analyzed by multiple analysis of variance in which habitat, subspecies, and distance were the treatments. A significant habitat effect would suggest that open and forest habitats differentially affected call degradation, and a significant subspecies effect would suggest that the calls of the two subspecies exhibited differential degradation. A significant interaction effect (habitat X subspecies) would suggest that the amount of call degradation for each subspecies was different in each habitat. The hypothesis proposed by Nevo and Capranica (1985) predicts that degradation of each subspecies is reduced in its own habitat relative to the foreign habitat; that is, an interaction effect should be apparent. A three way interaction between habitat, subspecies, and distance would suggest that the subspecies by habitat interaction was dependent on the distance from the source.

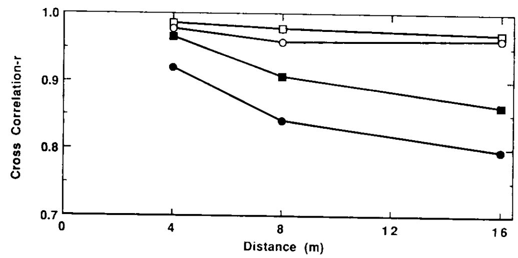

FIG. 1. The relation between the cross correlation coefficients, which estimate the amount of call degradation, and distance (squares, *A. c. crepitans*; circles, *A. c. blanchardi*; open, open habitat; closed, forest habitat).

RESULTS

As expected, the cross correlation coefficients decreased across distance, indicating increased degradation with increased distance from the source (Fig. 1). The calls of both subspecies exhibited less severe rates of degradation in the open habitat than in the forest. Both subspecies exhibited a decrease in the cross correlation coefficient of 0.015 in the open habitat from 4 m to 16 m, and analogous decreases of 0.103 and 0.123 (*A. c. crepitans* and *A. c. blanchardi*, respectively) in the forest habitat (Fig. 1). The calls of *A. c. crepitans* suffered less degradation than the calls of *A. c. blanchardi* in both habitats, but there is an interaction effect because the difference in the amount of degradation between the two subspecies was more pronounced in the forest than in the open habitat (Fig. 1).

The results of the multiple analysis of variance confirm the trends suggested by the graphical analysis. There were strong habitat, subspecies, and distance effects ($P < 0.001$). The habitat effect was of the greatest magnitude ($F = 134.2$, $df = 1{,}204$) with subspecies ($F = 23.8$, $df = 1{,}204$) and distance ($F = 23.6$, $df = 2{,}204$) affecting call degradation to a similar degree. Also, there was a significant interaction between subspecies and habitat ($F = 4.2$, $df = 1{,}204$, $P = 0.04$) owing to the greater difference in the amount of call degradation between the two subspecies in the forest than in the open habitat. The interaction between the three variables was not statistically significant; thus the interaction between subspecies and habitat is not distance dependent.

DISCUSSION

Our study offers support for the role of the environment in promoting call divergence between the two subspecies of *A. crepitans*. Ryan and Wilczynski (in press) rejected two alternative hypotheses for call differences: reproductive character displacement, and a correlated response to selection on body size.

The habitats in which the two subspecies reside differentially affect transmission efficiency, and thus the selection forces necessary to promote call divergence in the manner suggested by Nevo and Capranica (1985) are present. These authors suggested that the differences in the calls of the two subspecies would result in different effects on transmission efficiency, and in that too they were correct. Finally, they suggested that the calls of the two species would be adapted to enhance transmission efficiency in the local habitat. This "optimality" prediction of the environmental selection hypothesis is not supported because the calls of *A. c. crepitans* transmitted about as well or better than the calls of *A. c. blanchardi* in both habitats. Degradation was minimal in the open habitat but extreme in the forest. In this habitat, the difference between the subspecies was pronounced, with the calls of the native *A. c. crepitans* exhibiting less degradation than the calls of *A. c. blanchardi.* The significant subspecies by habitat interaction occurs because of the enhancement of the subspecies differences in the forest habitat. This suggests that environmental selection has played a significant role in call divergence between the two subspecies.

It is not surprising that transmission efficiencies were higher in the open habitat than in the forest. The forest is characterized by many stationary heterogeneities, such as trees and shrubs, that cause reverberation and thus increase degradation (Wiley and Richards, 1978, 1982). Reverberation is almost absent in open habitats. Although nonstationary heterogeneities, due to meteorological disturbances that are more common in open habitat, also contribute to degradation, Wiley and Richards (1978) suggest these effects are most pronounced during midday, not a time when most frogs vocalize or when these experiments were conducted. Our data show that there is strong environmental selection on calls in the forest but that this selection is relaxed and minimal in the open habitat.

This study was not designed to determine the acoustic basis of the subspecies differences in transmission efficiency. It is not clear why the call of *A. c. crepitans* transmitted more efficiently than the call of *A. c. blanchardi.* However, it is interesting to note that the call of *A. c. crepitans* is shorter, and when comparing two species of toads, Ryan and Sullivan (1989) also found that the signals of shorter duration exhibited less degradation.

Ryan and Wilczynski (1988, unpubl.) showed that both between and within populations of both subspecies of cricket frogs, females prefer lower frequency calls. Wagner (1989*a*, 1989*b*, 1990) showed that males producing lower frequencies and longer duration calls were more likely to win fights; the calls of *A. c. blanchardi* are both of lower frequency and longer duration than the calls of *A. c. crepitans.* Therefore, it appears that in both habitats calls may be under similar sexual and social selection, but only in the forest habitat are calls subjected to significant environmental selection. We suggest that the differences in calls between the two subspecies are due to release from environmental selection in the open habitat, resulting in a reduced constraint on the ability to respond to sexual and social selection.

Other data support the hypothesis that habitat influences the evolution of call structure. There is an isolated pine forest well within the range of *A. c. blanchardi* in central Texas. The calls of these forest frogs are statistically more similar to the calls of *A. c. crepitans* in forest habitat than they are to *A. c. blanchardi* in open habitat (Ryan and Wilczynski, in press), but allozyme analysis shows that these frogs are genetically more similar to *A. c. blanchardi* (Morizot, Ryan and Wilczynski, unpubl.). This suggests convergence of call structure due to habitat effects.

It would not be surprising if a single selection force did not account for the divergence of mate recognition signals within any species. Various causes of divergence in species-typical traits can interact, and across a species' range the relative importance of different selection forces could vary. Our data suggest this to be the case in *Acris crepitans,* where variation in the strength of environmental selection coupled with relatively constant sexual and social selection have led to the divergence of advertisement calls between subspecies.

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