

Asymmetric Assortative Mating between Two Hybridizing *Orchelimum* Katydid (Orthoptera: Tettigoniidae)

ABSTRACT.—The katydids *Orchelimum nigripes* and *O. pulchellum* (Orthoptera: Tettigoniidae) have a largely allopatric distribution in the eastern United States, but hybridization between these species has been well documented in two contact zones. I used animals from populations just outside a narrow, recently formed upstream-downstream hybrid zone along the Potomac River near Washington, D.C., in laboratory mate-choice trials to examine the possible importance of differential patterns of mate preference in determining the structure and dynamics of this hybrid zone. Offered a choice between a male of each species, *O. nigripes* females showed an extreme preference for conspecific mates, but *O. pulchellum* females showed no clear preference. This asymmetry in mate discrimination may contribute to the apparently ongoing replacement along the Potomac of *O. pulchellum* by *O. nigripes* in the wake of a moving hybrid zone.

INTRODUCTION

Behavioral interactions may facilitate or reduce hybridization between animal taxa. In acoustic Orthoptera, for example, interspecific differences in the calling songs of different species have often been emphasized in the literature as extremely important reproductive isolating mechanisms, effectively preventing interspecific mating (*e.g.*, Otte, 1992, 1994). In most katydids and crickets, calling songs are produced by males, and females are attracted to them. An extensive body of experimental work has demonstrated that females of many species exhibit a strong phonotactic preference for conspecific song, as well as a preference for conspecific songs with certain characteristics (*e.g.*, Ewing, 1989; Bailey, 1991). Much emphasis has been appropriately and productively placed on studying the importance of the calling song in mate selection, but most such studies have relied on measuring the response by females to electronic playbacks of natural or manipulated songs under acoustically pristine laboratory conditions. Although this approach allows great experimental precision, mate choice experiments using only recorded songs rather than live males necessarily ignore interactions between potential mates subsequent to initial attraction. These postattraction interactions may be critically important in determining whether or not copulation, gamete transfer and fertilization actually occur (this is especially likely for taxa in which individuals spend considerable time assessing potential mates before accepting or rejecting them).

Orchelimum nigripes and *O. pulchellum* are closely related, but morphologically and genetically distinct, conocephaline katydids that are widespread and abundant in the eastern United States. Their distribution is largely allopatric (Morris and Walker, 1976; Shapiro, 1998), and based on extensive field experience with both taxa, no differences in habitat or other aspects of their ecology are apparent. Some time within the last 75 y *O. nigripes* became established within the Potomac River basin above Washington, D.C., where it is currently very abundant, having completely replaced *O. pulchellum* (the typical species east of the Appalachians) along the river corridor east of the Appalachians and north of the city. These two katydids now form a narrow upstream-downstream hybrid zone where they meet along the Potomac River in the vicinity of Washington, D.C. (Shapiro, 1998).

Both field and laboratory observations have shown that female *Orchelimum* often spend substantial amounts of time in close proximity to singing males, appearing very attentive and often engaging in physical contact, before either copulating or moving away (Feaver, 1983; pers. obs.). These observations suggest that close-up assessment of prospective mates may be important in these katydids. Such interactions are likely to be especially important for *O. nigripes* and *O. pulchellum* because the songs of these two taxa are extremely similar, perhaps identical, differing, if at all, only in a minor aspect of phonotome structure (G. K. Morris, pers. comm.). Indeed, preliminary phonotaxis studies found no evidence that females distinguish between songs of the two taxa, although they did discriminate against the songs of other congeners (Shapiro, 1996). In the present study, I used *O. nigripes* and *O. pulchellum* from the vicinity of the Potomac hybrid zone to investigate the potential influence of assortative mating in structuring this contact zone. To look for evidence of species discrimination I carried out laboratory

TABLE 1.—Results of mate choice trials. Results are shown separately for 1994 and 1997 in parentheses (1994, 1997), and were analyzed together using the Mantel-Haenszel test for replicated 2×2 tables (Sokal and Rohlf 1995). *Orchelimum nigripes* females were significantly more discriminating than *O. pulchellum* females ($X^2_{MH [1]} = 4.94$, $P = 0.0262$)

Female	Male mate	
	Conspecific	Heterospecific
<i>O. nigripes</i>	21 (11, 10)	1 (0, 1)
<i>O. pulchellum</i>	14 (8, 6)	8 (3, 5)

choice trials using live animals in order to avoid the common experimental problem of excluding short-range mating cues.

METHODS

I conducted mate choice trials in 1994 and 1997. Females were collected as virgins (late instar nymphs) from *Orchelimum nigripes* or *O. pulchellum* populations in the Potomac region that were "pure" based on genetic and morphometric studies (Shapiro, 1998); males were collected from these same sites. Animals were maintained in the laboratory and females were used in mating experiments approximately 2 wk following the final moult (most between 12 and 16 d; all between 10 and 25 d).

For each trial, a female *Orchelimum nigripes* or *O. pulchellum* was placed in a 12" \times 12" \times 12" wooden cage, enclosed by fine mesh on all sides. One *O. nigripes* and one *O. pulchellum* male were then added to the cage. Males of both species attempted to mate with females of both species, and whether or not courting males were eventually able to copulate with a female was clearly a matter of female choice, although some individual males courted more aggressively than others. If a copulation occurred within 60 to 70 min, the unsuccessful male was removed shortly after initiation of the copulation. When the copulating pair eventually separated, the female was placed in an oviposition cage and not reused in mating trials. A total of 44 copulations resulted from these trials, each copulation involving the transfer of a spermatophore, including a large spermatophylax, which the female consumed after decoupling. Most males were used in only a single trial; however, because the number of males available for experiments was limited, six *O. nigripes* and six *O. pulchellum* males were used twice, once with an *O. nigripes* female and once with an *O. pulchellum* female. These males were given a minimum of 72 h between a copulation and a second trial. This is sufficient time for males to remate, and males that were reused showed no evidence of reduced mating success in their second trials, so all trials were treated as independent. For successful matings I recorded the time elapsed between the introduction of the two males to the mating arena and the initiation of copulation (latency to copulation) as well as the duration of copulation.

RESULTS

Orchelimum nigripes females offered a choice between an *O. nigripes* male and an *O. pulchellum* male chose the *O. nigripes* male in 21 of 22 copulations (95%; cumulative binomial probability (one-tailed) for $p = q = 0.5$: $P = 5.4836 \times 10^{-6}$). However, *O. pulchellum* females offered this choice failed to show such clear discrimination. *Orchelimum pulchellum* females chose the *O. pulchellum* male in just 14 of 22 copulations (64%; cumulative binomial probability (one-tailed) for $p = q = 0.5$: $P = 0.1431$). Overall, *O. nigripes* females were significantly more discriminating than *O. pulchellum* females (Table 1; Mantel-Haenszel test for replicated 2×2 tables, with continuity correction: $\chi^2_{MH [1]} = 4.94$, $P = 0.0262$ (Sokal and Rohlf, 1995)).

Data on latency to copulation were not normally distributed. However, log-transformed data were not significantly different from normal, so these transformed data were used in ANOVA. Because only a single *Orchelimum nigripes* female mated with an *O. pulchellum* male, analyses of latency to copulation and copulation duration were limited to the three remaining types of matings. An ANOVA showed no difference among the three types of matings for either latency to copulation or copulation duration (Fig. 1).

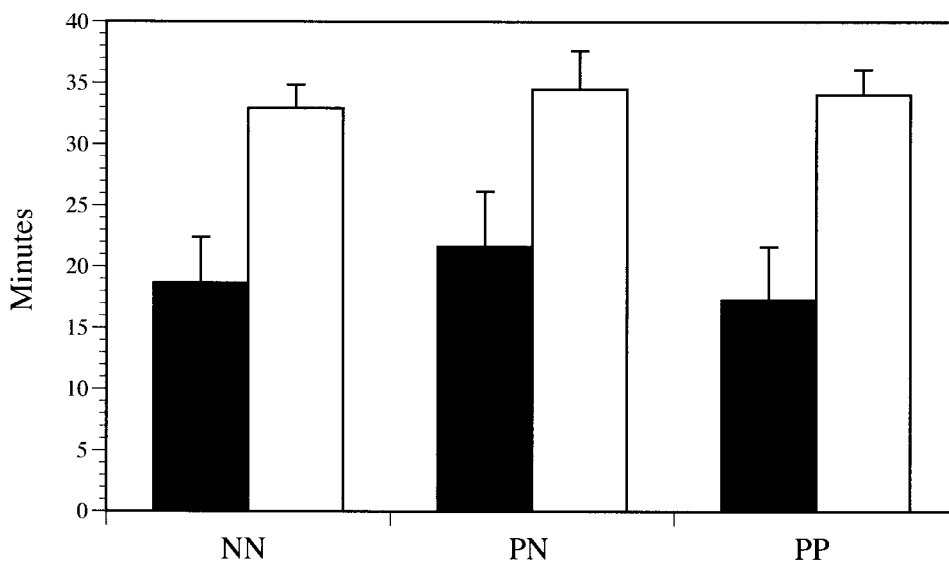


FIG. 1.—Mean latency to copulation (black) and mean duration of copulation (white), with standard error bars, for matings between *Orchelimum nigripes* (N) and *O. pulchellum* (P). NN and PP are conspecific matings of *O. nigripes* ($n = 21$) and *O. pulchellum* ($n = 14$), respectively. PN matings ($n = 8$) involve an *O. pulchellum* female and an *O. nigripes* male. There was only a single mating between an *O. nigripes* female and an *O. pulchellum* male, so this class of matings was excluded from this analysis. The three remaining types of matings do not differ for either latency to copulation (ANOVA on log-transformed values: $F_{2,40} = 0.77$, $P = 0.4705$) or copulation duration (ANOVA: $F_{2,40} = 0.13$, $P = 0.8790$). Displayed latency data are untransformed means and standard errors

DISCUSSION

This study revealed a striking asymmetry in mate discrimination between female *Orchelimum nigripes* and *O. pulchellum*. Female *O. nigripes* showed a dramatic preference for mating with conspecific males, but there was no clear evidence of any preference by *O. pulchellum* females for males of either species. Asymmetry in the success of reciprocal interspecific (or interpopulation) crosses has been described for several other taxa. Often the reported asymmetry has involved only postmating mechanisms differentially affecting reciprocal crosses, with no apparent premating isolation for either reciprocal cross (*e.g.*, field crickets: Harrison, 1983; grasshoppers: Bella *et al.*, 1992; waterstriders: Gallant and Fairbairn, 1997). For a few taxa, however, asymmetry in premating isolation, or in both premating and postmating isolation, has been described (*e.g.*, waterstriders: Spence, 1990; Zimmerman and Scholl, 1993; allopatric populations of flour beetles: Wade *et al.*, 1995; allopatric stickleback populations: Ayvazian, 1993; pocket gophers: Patton and Smith, 1993).

Asymmetric reproductive isolation can be important in shaping the dynamics of hybrid zones. If two initially geographically isolated taxa come into contact, and females of one taxon produce a greater proportion of pure offspring than do females of the other taxon, then in subsequent generations the first genome will tend to replace the second, *i.e.*, the position of the hybrid zone will shift (*cf.*, Bella *et al.*, 1992). Such a mechanism may partly explain apparent historical movement of a hybrid zone involving two waterstriders that exhibit asymmetric sexual isolation (Sperling and Spence, 1991; Klingenberg *et al.*, 2000). In the Potomac *Orchelimum* hybrid zone, geographic patterns of genetic and morphological variation, combined with historical information on *Orchelimum* distribution patterns in the region, strongly suggest that such a shifting of the hybrid zone has occurred here as well (Shapiro, 1998), and it is possible that asymmetric mate preferences have contributed to this movement (see below).

Results from the present study raise the question of what proximate mechanisms are used by these katydids in close-up species discrimination. Several likely possibilities warrant future investigation. For example, these insects are very visually aware and brightly colored, differing especially conspicuously in tibial coloration (jet black in *Orchelimum nigripes*, red-brown in *O. pulchellum*), which might provide an effective cue for mate recognition. Chemical cues could also be important in mate recognition (e.g., Bell, 1980; Ritchie, 1990; Neems and Butlin, 1995; Tregenza and Wedell, 1997); although no such cues have yet been documented for any katydid, *Orchelimum* male-female interactions often involve extensive mutual tapping and stroking with their antennae. Identifying specific factors important in mate choice will require detailed observations of interacting males and females and manipulative experiments (e.g., mating trials using males with painted legs).

As noted above, the asymmetry in mate discrimination by Potomac *Orchelimum nigripes* females versus Potomac *O. pulchellum* females suggests a possible mechanism that could contribute to the replacement of the native *O. pulchellum* by *O. nigripes* along the Potomac River, a process that is likely ongoing (Shapiro, 1998). Interspecific crosses from these populations have been shown in the laboratory to produce viable offspring, although heterospecifically mated females suffer substantially reduced fecundity (Shapiro, 2000) and F₁ hybrid males may be sterile (Cabrero *et al.*, 1999). If female *O. nigripes* discriminate strongly against non-*nigripes* phenotypes, they will continue to produce pure *O. nigripes* progenies. In contrast, if *O. pulchellum* females mate indiscriminately with *O. nigripes*, with *O. pulchellum* and perhaps with males of mixed ancestry, their progenies will represent a mix of the *O. nigripes* and *O. pulchellum* genomes. Such a pattern would contribute to a net flow of *O. nigripes* genes into the *O. pulchellum* genome and hence movement of the hybrid zone toward *O. pulchellum* (although this effect might weaken with an increasing proportion of phenotypically variable backcrossed individuals in the population). Furthermore, heterospecifically mating *O. pulchellum* females may incur a significant fitness cost in the form of reduced fecundity and the production of sterile hybrid males, which could also contribute to an advance of the *O. nigripes* front.

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LEO H. SHAPIRO¹, Department of Ecology and Evolution, State University of New York, Stony Brook 11794-5245.

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¹ Present address: Insect Biology, ESPM, 201 Wellman Hall 3112, University of California, Berkeley 94720