Geographic variation in hybrid fertility in the field crickets *Gryllus integer*, *G. rubens*, and *Gryllus* sp.

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Hybridization was attempted between three geographically separated field cricket populations having males that produce distinct songs. *Gryllus integer* from central Texas and *G. rubens* from Arkansas produced many offspring in both reciprocal crosses. *Gryllus* sp. from New Mexico also produced hybrid offspring in matings with *G. integer* and *G. rubens* but at a very low frequency and only in crosses where the female was *Gryllus* sp. In previous experiments, *G. integer* from California was unable to hybridize with *G. integer* from Texas and *G. rubens*.

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Des expériences d'hybridation ont été tentées entre trois populations géographiques de grillons à chants différents. Les croisements réciproques entre *Gryllus integer* du centre du Texas et *G. rubens* d'Arkansas ont donné de nombreux rejetons. *Gryllus* sp. du Nouveau-Mexique a également produit des hybrides lors de croisements avec *G. integer* et *G. rubens*, mais avec une fréquence très faible et seulement lorsque les femelles étaient des *Gryllus* sp. Au cours d'expériences antérieures, les croisements entre des *G. integer* de Californie et des *G. integer* du Texas ou des *G. rubens* n'avaient pas donné d'hybrides.

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Introduction

The rate at which pulses of song are produced and the grouping of pulses into chirps or trills are species-specific characteristics of cricket calling songs and are used to identify species (Alexander 1957, 1962; Otte *et al.* 1987). In the southern United States, however, the taxonomy of field crickets producing three distinct trilling songs is not clear. *Gryllus integer* in California produces a so-called stutter–trilling song with pulses of sound arranged in groups or chirps of two or three at 700–1400 chirps/min and 43–70 pulses/s at 25°C. Texas *G. integer* has a more continuous trilling song, a highly variable number of pulses in a burst or trill, and a pulse rate of 70–90/s at 25°C. *Gryllus rubens* is found from east Texas to Florida and has a continuous trilling song similar to Texas *G. integer* but at a pulse rate of 46–64/s at 25°C (Nickle and Walker 1974; Smith and Cade 1987; Weissman *et al.* 1980). The name *G. integer* has been used routinely to refer to individuals producing the Texas and California songs. Texas and California populations are referred to here as *G. integer* (Tex.) and *G. integer* (Cal.).

In previous experiments all crosses between *G. rubens* and *G. integer* (Tex.) were as fertile as conspecific matings, but crosses between *G. integer* (Cal.) and *G. integer* (Tex.) or *G. rubens* were infertile (Smith and Cade 1987). Cultures in these experiments were established for both types of *G. integer* from widely separated populations in Davis, CA, and Austin, TX. *Gryllus rubens* was from McAlester, OK, very close to an area of overlap with *G. integer* (Tex.) (Walker 1974). The geographical distance between *G. integer* (Cal.) and *G integer* (Tex.) or *G. rubens* and associated ecological factors may contribute to hybrid sterility by reducing the probability of interbreeding between these populations (Templeton 1989; Hewitt 1989). The purpose of the present study was to test the hypothesis that the geographic distance between parental populations is, in part, responsible for the failure of *G. integer* (Tex.) and *G. integer* (Cal.) to hybridize. Hybridization was attempted using individuals from a population of trilling field crickets from Las Cruces, NM, a location that is approximately half way between the California and Texas *G. integer* populations studied previously. The taxonomic relationship between the Las Cruces population and *G. integer* (Cal.) and *G. integer* (Tex) is unknown, and the Las Cruces population is referred to here as *Gryllus* sp. Also, hybrid crosses were performed between a new population of *G. rubens* from Arkadelphia, AK, *G. integer* (Tex.), and *Gryllus* sp. to study geographic variation in the ability of *G. rubens* to hybridize with the other populations.

Methods

Study animals

Specimens of trilling *Gryllus* were collected in Arkadelphia, Austin, and Las Cruces in 1986 and laboratory cultures were established. Tape recordings and sonagrams were made of the songs of several males from each type of culture to ensure the identification of *G. integer* (Tex.) and *G. rubens* and to compare the song of *Gryllus* sp. with the other species. See Smith and Cade (1987) for more details on cricket cultures, tape recordings, and sonagrams.

Hybridization experiment

Individual adults were placed in 500-mL plastic containers with food and water. At 5 d after eclosion, one male and one female were placed in a mating chamber (28 × 18 × 12 cm) that contained food, water, and a 500-mL dish with approximately 200 mL of a fine sand and vermiculite mixture for oviposition. Five pairs of all possible crosses were established in this manner including the following: three conspecific crosses that served as controls and the experimental crosses of *G. integer* (Tex.) male × *Gryllus* sp. female, *Gryllus* sp. male × *G. integer* (Tex.) female, *G. integer* (Tex.) male × *G. rubens* female, *G. rubens* male × *G. integer* (Tex.) female, *Gryllus* sp. male × *G. rubens* female, and *G. rubens* male × *Gryllus* sp. female. Crickets were not observed for mating behavior. Pairs were maintained until they died or until 7 d after nymphs first appeared. Newly hatched nymphs were counted and removed from oviposition dishes daily. After 60 d the oviposition soil was frozen for later examination for unhatched eggs. This period is long enough for all fertile eggs to hatch (Backus 1985). Unhatched eggs were removed by first washing the oviposition soil in a 40% zinc sulphate solution that caused the eggs to float (Destephano *et al.* 1982). Three separate washes were made and the eggs were counted. The oviposition soil was then examined under 40× magnification and the remaining unhatched eggs were counted.

Results

Representative sonagrams of the songs produced by males from *G. integer, G. rubens,* and *Gryllus* sp. are in Fig. 1.

Hybridization experiment

The average number of eggs (nymphs + unhatched eggs) and nymphs and the percentage of eggs that hatched from the various crosses are in Table 1. All three control crosses produced large numbers of eggs and offspring. *Gryllus* sp. produced significantly more eggs than *G. integer* (Tex.) (Mann–Whitney, $U = 5.0$, $p = 0.04$), but the fertility of these control groups did not differ significantly ($U = 8.0$, $p = 0.1$). There were no significant differences in the number of eggs or fertility between *G. integer* (Tex.) and *G. rubens* ($U = 6.0$, $p = 0.06$ in both comparisions). Also, *G. rubens* and *Gryllus* sp. did not differ significantly in the number of eggs produced ($U = 12$, $p = 0.5$) or in fertility ($U = 6$, $p = 0.1$).

Hybridization between male *G. integer* (Tex.) and female *Gryllus* sp. and between male *G. rubens* and female *Gryllus* sp. produced offspring, although the number of eggs and fertility were much lower than in the controls. No offspring were produced in the two hybrid crosses with a male *Gryllus* sp., although the number of eggs produced was roughly equivalent to the reciprocal crosses. Both crosses involving *G. integer* (Tex.) and *G. rubens* produced many eggs and offspring.

Discussion

The sonagrams for *G. integer* (Tex) and *G. rubens* used here are very similar to those published previously and confirm identification of these study animals (Smith and Cade 1987). The song of *Gryllus* sp. is different from the other two species in having a lower frequency and fewer pulses per burst of song. Within each burst, the pulses are arranged in smaller grouping of two or more. *Gryllus* sp. also has shorter bursts of song and is more variable in number of pulses in groups than *G. integer* (Cal.). More information is necessary on the *Gryllus* sp. songs, but the song differences observed here are of the type usually invoked to separate species (for examples, see Otte *et al.* 1987). Alternatively, it is possible that a cline exists between California and Texas along which the song of *G. integer* (Tex.) becomes more similar to that of *G. integer* (Cal.).

The *G. integer* (Tex.) control crosses produced significantly fewer eggs than *Gryllus* sp. controls, but all other comparisons between controls were not significantly different. The difference in egg production between *G. integer* (Tex.) and *Gryllus* sp. could be species specific or reflect the laboratory strains used in this experiment. In any case, the number of eggs, number of nymphs, and fertility were within the range observed previously for these and other *Gryllus* species (Bigelow 1960; Harrison 1983; Simmons 1988; Smith and Cade 1987).

Hybrid crosses between *G. integer* (Tex.) and *G. rubens*

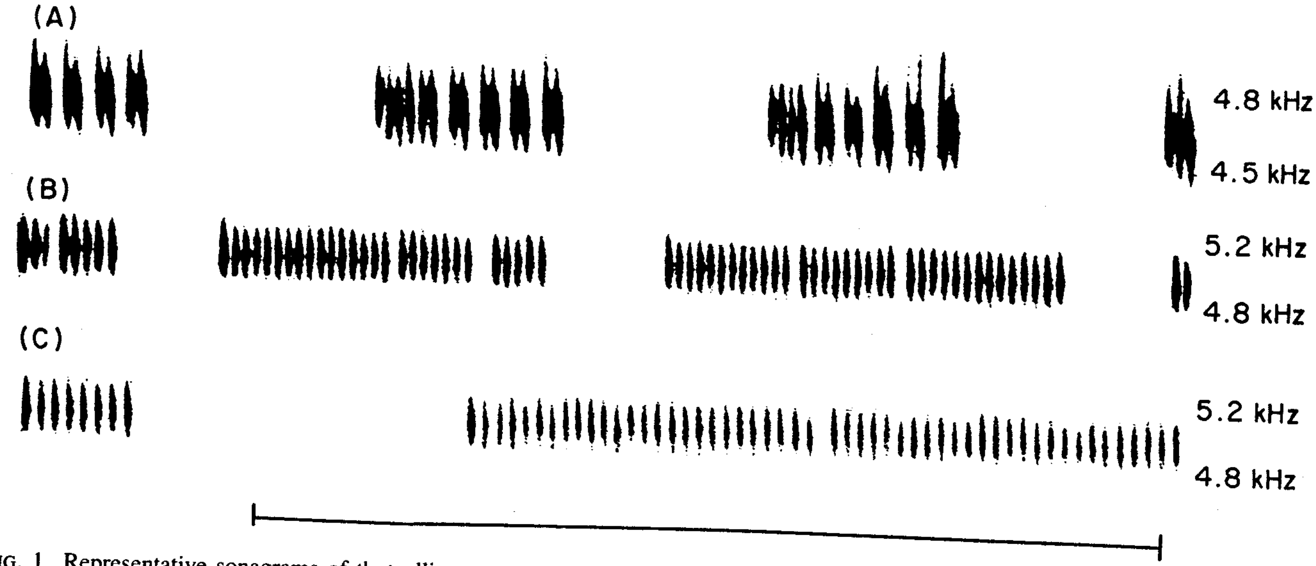

FIG. 1. Representative sonagrams of the calling songs of male (A) *Gryllus* sp., (B) *G. integer* (Tex.), and (C) *G. rubens*. Midranges of frequency are shown. The scale line represents 1 s.

TABLE 1. The average number of total eggs produced, the number of nymphs hatching from these eggs, and the percent fertility (nymphs/total eggs) for each of the control and experimental crosses

Mating pairs	No. of eggs	No. of nymphs	% hatched
Control crosses			
G. integer (Tex.)	363.2 (333.8)	159.5 (162.2)	43.8 (32.1)
Gryllus sp.	617.2 (229.5)	378.6 (140.3)	62.9 (16.2)
G. rubens	646.8 (235.9)	497.4 (214.1)	76.5 (34.4)
Experimental crosses (male × female)			
G. integer (Tex.) × *Gryllus* sp.	249.6 (159)	29.2 (65.3)	7.4 (16.5)
Gryllus sp. × *G. integer* (Tex.)	215.8 (55.7)	0	0
Gryllus sp. × *G. rubens*	269.2 (129.2)	0	0
G. rubens × *Gryllus* sp.	162.4 (167.4)	79.8 (178.4)	17.8 (39.8)
G. integer (Tex.) × *G. rubens*	193 (69.3)	151.4 (86.3)	69.1 (39.4)
G. rubens × *G. integer* (Tex.)	353.6 (253.5)	253.6 (250.3)	52.4 (48.3)

NOTE: Values in parentheses are standard deviations.

were very fertile. This result confirms the previous finding that matings between *G. integer* (Tex.) and *G. rubens* from Oklahoma were as fertile as conspecific matings (Smith and Cade 1987). By contrast, *Gryllus* sp. produced eggs and offspring at low frequencies when paired with *G. integer* (Tex.) and *G. rubens* and only in crosses where the female was *Gryllus* sp. The ability to hybridize successfully was, however, in contrast with previous experiments using *G. integer* (Cal.), and *G. integer* (Tex.) and *G. rubens* where no offspring were produced. The greater proximity of *Gryllus* sp. to *G. integer* (Tex.) and *G. rubens* may contribute, in part, to hybrid fertility by increasing the probability of gene flow between populations.

Harrison (1983) also found asymmetries in fertility between hybrid crosses of *G. firmus* and *G. pennsylvanicus*. Hybrid offspring were produced when the male was *G. firmus*, but rarely when the male was *G. pennsylvanicus*. Such asymmetries may result from the failure to mate successfully if the male is of one species, a form of prezygotic isolation. Also, hybrid nuclei may only develop in the egg cytoplasm of one type of female. In the present experiment it is not known if hybrid sterility involving male *Gryllus* sp. and female *G. integer* (Tex) and *G. rubens* resulted from failure to mate, to transfer sperm, or postzygotic factors. The number of eggs produced in these two hybrid crosses was approximately the same as in the hybrid crosses where the female was *Gryllus* sp. This observation suggests that male *Gryllus* sp. did mate in pairings with female *G. integer* (Tex.) and *G. rubens* since male crickets transfer egg development stimulants to the female in the spermatophore (Loher and Edson 1973).

More information is needed on song structure as well as the degree of hybrid fertility in field cricket populations from California to Florida to understand better the biosystematics of this group.

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