Genetic Control of Song Specificity in Crickets

Abstract. *The calling song of male field crickets is composed of stereotyped rhythmic pulse intervals, which are predictable expressions of genotype. Females identify conspecific males by their song. Two species of crickets were found to exhibit species-specific song preference, and hybrids between them preferred hybrid calls over either parental call. These results imply genetic control of song reception as well as transmission.*

Acoustical communication in field crickets is extremely important to these nocturnally active species. The stereotyped calling song of adult male field crickets attracts females (which do not sing) for mating (*1*, *2*). In previous experiments, measurements were made of the ability of a female cricket to orient toward the sound by means of tympana on her forelegs and to walk to it. We were able to quantify the phonotaxis by measuring the "phonomotor response," a cricket's turning tendency while it is walking in place on a Y-maze in a sound field. Our experimental paradigm is the optomotor studies (*3*, *4*).

Teleogryllus commodus and *T. oceanicus* are northern and southern Australian field crickets, respectively, which overlap geographically in southern Queensland. These crickets differ markedly in rhythmic structure of their calling songs. Exhaustive measurements of rhythmic pulse intervals show that these two species produce viable hybrids whose calling song is distinctly different from either parental song. Most rhythmic elements of hybrid song are intermediate between parental ones; this suggests polygenic control (*5*, *6*). We showed that genetic differences that cause song changes in males also apparently alter responsiveness to song in females.

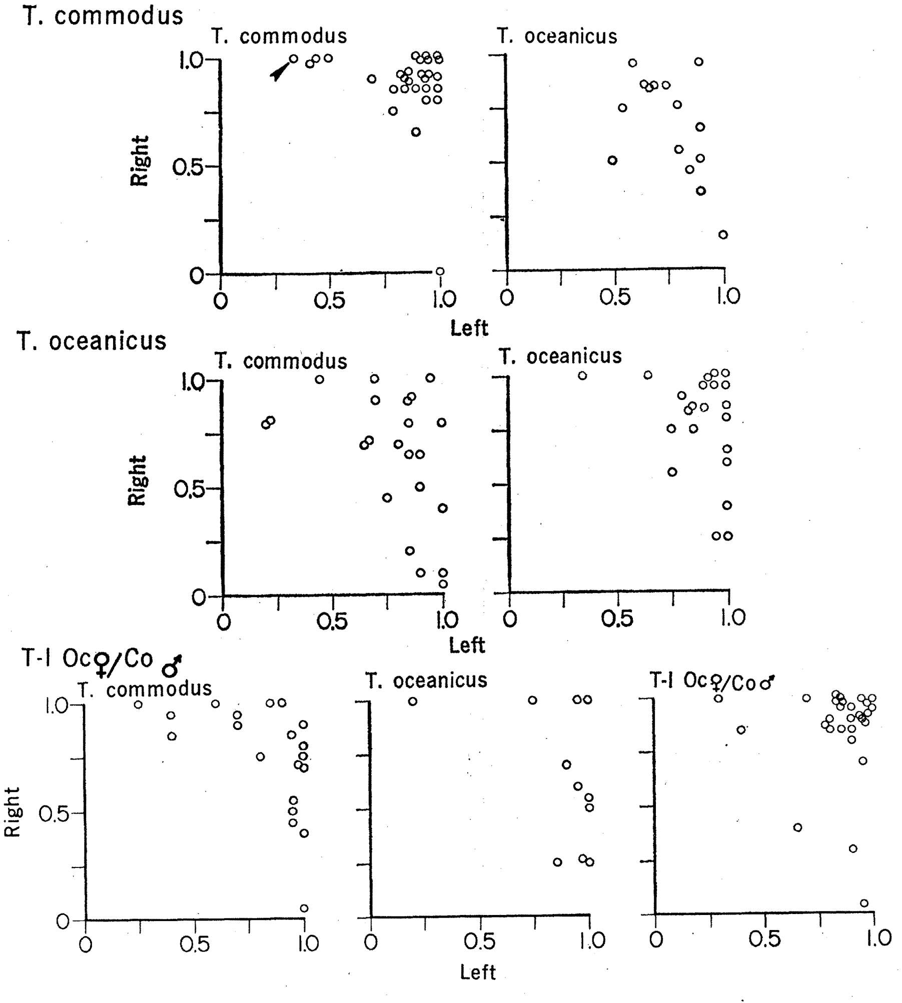

Fig. 1. Phonomotor performance (r) of *Teleogryllus* species in response to conspecific and heterospecific calling songs. Top scattergrams refer to the performance of *T. commodus* when played recorded songs of *T. commodus* and *T. oceanicus*. Middle scattergrams show performance of *T. oceanicus*. The bottom set refers to performance of hybrid females (formed from a cross of *T. oceanicus* females and *T. commodus* males) when played recorded calling songs of both parental species as well as that of sibling male hybrids. Each scattergram summarizes phonomotor performance as a function of sound from the cricket's right and left. Each point represents a different animal. The r value for left-directed sound can be read from the horizontal axis, and that for right-directed sound can be read from the vertical axis. This graphic representation does not convey a functional relationship between r values for right and left, which vary independently of each other.

We measured locomotor response to sound by placing tethered females upon a Y-maze globe, which they suspended in midair (see cover). This styrofoam maze consisted of three straight run paths of 10.5 mm interconnected by two Y choice points. Recordings of cricket song were played from symmetrically placed high-fidelity speakers (Daltronix) on a female's left and right; each speaker was situated 94 cm from the maze and deviated from her longitudinal body axis by 40°. Although her position in space with respect to each sound source was fixed, she could walk freely on the maze (which rotated in space beneath), and when she inevitably came to a choice point she had to choose the right or left arm of the maze. Her behavior at choice points with respect to the sound source was measured by her turning tendency, which we termed r and defined as the number of turns toward the sound source divided by the total number of turns.

Sound stimuli were provided by playing tape loops of recorded calls. In a typical experiment sound was played through either the right or left speaker for 20 choices, whereupon the origin of the sound was immediately switched to the opposite speaker for another 20 choices. A Uher 4400 tape recorder was used both to record and play back calls. Audiospectrograms of the tape-recorded calls were made on a Kay 7030 sonograph, and the frequency spectra of the calls agreed with data of LeRoy (*6*) for *T. commodus* and *T. oceanicus*. The intensity of the calls measured at the maze was 69 db relative to 2×10^{-4} μbar. The 147 adult, virgin females used in these experiments varied in adult age from 1 to 6 weeks; older females were less likely to run on a maze. Hybrid crickets (T-1) resulted from crossing virgin *T. oceanicus* females with *T. commodus* males; several hundred offspring grew to adulthood, and 60, isolated at the final instar, were studied. All experiments were performed in an anechoic chamber (*7*) at 24° ± 2°C. We recorded calling songs at several different temperatures so that we could match recording and playback temperatures within 2°C. All animals supported the maze when it was presented, but they usually would not walk unless stimulated acoustically.

Each female was required to make 40 choices, 20 with sound played from the right speaker and 20 from the left speaker. An r value (turns toward the sound divided by 20) was obtained for each speaker. Values of r could be between 0 and 1.0; $r = 0$ if no turns were toward the sound, $r = 1.0$ if all were toward the sound, and $r = 0.5$ if there was no preference. The r values for right and left sound directions are displayed conjointly in each scattergram of Fig. 1. Each point represents the performance of a different, individual female for 20 choices in each direction. The relative clustering or scattering of data points can be seen.

We arbitrarily defined a strong phonomotor response as one having values of 0.75 or greater (15 of 20 turns directed toward sound) for each of the sound directions. By this standard, maze behavior can be explained in terms of species-specific song preference (Table 1). This is also seen when the clustering of data points for homospecific song is compared to the relative scatter of points for heterospecific song. Statistical significance was demonstrated by applying the G-test of independence. Preference for homospecific song is significant at the 1 percent confidence level in all three groups. Thus, in the phonomotor experiment, as in previous phonotaxis experiments, female crickets are attracted to homospecific calling song. Also, hybrid females show an equally strong preference for the calling song of hybrid males over either parental call. To our knowledge this is the first such demonstration in field crickets (*8*).

The arrow in Fig. 1 shows that some animals turned consistently in one direction regardless of the direction of sound. Thus one *T. commodus* animal turned to the right in all choices with sound from the right, and when sound was switched to the left the animal continued to turn right in 70 percent of choices. We have evidence (*9*) that such animals are "right-biased" (or "left-biased") and turn right (or left) when presented nondirectional sound; such locomotor bias was previously observed in an optomotor experiment (*4*). To exclude biased animals from our sample, we chose the symmetrical criterion of r values of 0.75 or greater for both right and left sound directions (Table 1).

Table 1. Comparison of phonomotor response of 147 female crickets to conspecific and heterospecific calling songs. The criterion performance was $r \geqq 0.75$ both for sound played from the right and for sound from the left (20 choices for direction).

Calling song	Females at criterion/ total tested	Females at criterion (%)
	T. oceanicus *on maze*	
T. oceanicus	14/22	63.6
T. commodus	4/22	18
	T. commodus *on maze*	
T. oceanicus	3/15	20
T. commodus	21/28	75
	Hybrid (T-1) on maze	
T. oceanicus	3/11	27.3
T. commodus	8/21	38
Hybrid (T-1)	21/28	75

Female hybrids can not only identify the song of the sibling hybrid males, but find it more attractive than parental song. This has interesting physiological implications. Perhaps female crickets carry genetically conferred sensory templates (*10*) of the species calling song. Genetic hybridizations would result in hybrid templates that are revealed only in song discriminations, since females do not announce their genetic identity as do calling males. What are some possible features of this template? It must contain information about the rhythmic elements of the song, since frequency modulation does not occur in cricket songs (*11*), although sensory elements may be tuned to the carrier frequency of their species song (*12*). This raises the question of the nature of the "species filter," particularly at the level of neurophysiological mechanisms. Are such filters single cells or networks of several cells? The capacity of the nervous system to abstract species-specific features of acoustical calling signals has been demonstrated in the bullfrog, and the existence of mating call detectors has been proposed (*13*). Although it is premature to speculate on the neuronal loci under genetic control in crickets, coupling of the male's song generator and a female's species-specific sensory template through a common set of genes presents an attractive mechanism for speciation in crickets and is relevant to the evolution of communication in these species (*11*).

We believe that the phonomotor technique is a valid measure of a female's normal acoustical response, that is, of phonotaxis. The two techniques are not exactly comparable because a tethered cricket on the maze maintains a fixed angle with respect to a constant-intensity sound source, whereas the free-walking animal in a phonotaxis experiment, once she localizes the sound source, usually turns to directly face the source and runs straight toward the increasingly louder signal (*2, 9*). The phonomotor method allows us to measure the relative attraction of one song compared to another. If the species-specific song is not presented, however, the cricket often will be attracted to heterospecific songs; they do not simply ignore or avoid songs of other species, and the same is true of free-walking animals (*9*). This technique is not limited to behavioral measurements. It should be feasible to record from neurons during the maze behavior. Neural units that code information about rhythmic elements of song have been described (*14*), and they have been monitored during apparently normal behavior of crickets (*15*). Coupled with further genetic experiments and neurophysiological analysis, such efforts should facilitate our understanding of the genetic control of behavior.

RONALD R. HOY
ROBERT C. PAUL

Department of Biology,
State University of New York,
Stony Brook 11790

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