Hybrid Cricket Auditory Behavior: Evidence for Genetic Coupling in Animal Communication

Abstract. *Male field crickets produce species-specific and stereotyped calling songs. Conspecific females are attracted to the call. Reciprocal F_1 hybrid females prefer the calls of sibling hybrids to reciprocal hybrids. Discrimination is probably based on temporal pattern and not carrier frequency of the call. The results imply that production of song by males and its detection by females have a common genetic basis.*

Interest in animal communication has recently turned to the question of genetic determinants in such behavior. Insects, and particularly crickets, have been studied from both genetic and communication viewpoints because their species-specific auditory behavior is highly stereotyped and predictable and because interspecific hybrids can be made in the laboratory (*1*). Detailed analyses have been made for two species of Australian field crickets, *Teleogryllus commodus* and *T. oceanicus*, and their hybrids. Measurements of the temporal pattern of the male calling song (only males sing) indicate it is genetically determined (*2–5*). Auditory behavior of the female is measured by her attraction to calling song (phonotaxis), and it, too, is strongly influenced by genotype (*6*). Reciprocal matings between *T. commodus* and *T. oceanicus* produced two F_1 hybrid types and each type sang distinctly different calls from the other type, and from either parental species (*5*). We now report that females of the two F_1 hybrid types discriminate between the F_1 calls; female hybrids prefer the call of their hybrid "brothers" over the reciprocal type. This reinforces the inference that both sender and receiver are genetically, as well as behaviorally, coupled in their auditory behavior.

The two classes of F_1 hybrids, T-1 and T-2, were produced by reciprocal matings between *T. oceanicus* and *T. commodus* and are defined as follows: T-1 is *T. oceanicus* ♀ × *T. commodus* ♂; T-2 is *T. commodus* ♀ × *T. oceanicus* ♂. Thus, the hybrids differ simply in maternal and paternal species; we will use "sibling" for male and female F_1's of a common cross type, and "reciprocal" for individuals of the other cross type. Our study was conducted at two different laboratories (*7*) and will be referred to as series A (1973) and series B (1975).

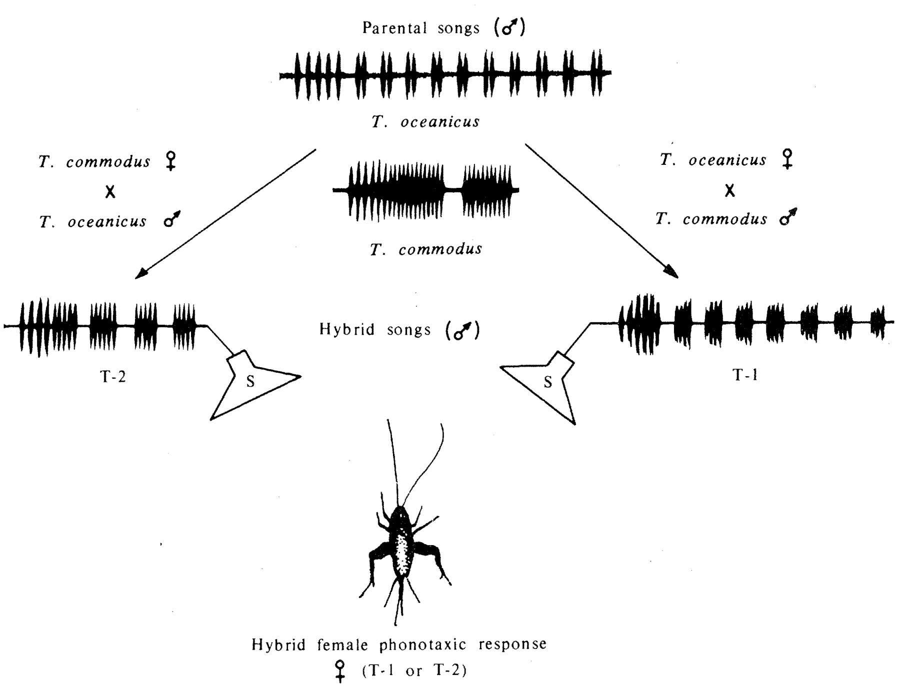

Fig. 1. Diagram of phonotaxis experiment. Adult female hybrid crickets chose between T-1 and T-2 hybrid calling songs, played simultaneously through separate speakers, by walking to one or the other speaker. Her choice was between the call of her own male siblings, or that of male siblings from the reciprocal cross. Once a female made a choice she was never retested. The temporal structure of the two hybrid calls is different, as indicated by oscillograms made from tape recordings of the calls. Oscillograms showing the temporal structure of parental calls, *T. commodus* and *T. oceanicus*, are shown for purposes of comparison; parental calls were not played to hybrid females in this experiment. *S*, speaker.

We made auditory discrimination tests by allowing freely walking females to choose between T-1 and T-2 songs. Females were isolated from males in postembryonic nymphal stages before auditory behavior develops, to minimize prior auditory experience. Each female was put into a small screened container and placed on the gravel-covered floor of an arena (1.3 by 1.4 m) in a soundproof anechoic room. The room was illuminated by dim red light for observation. Tape-recorded calling songs of T-1 and T-2 were played simultaneously through separate channels (and speakers) of a stereo tape recorder (*8*). The carrier frequency of calls was measured with a Kay 5029 sonograph, and measurements of interpulse intervals in the call revealed its temporal structure; in both respects they were typical of hybrid songs (*2, 5*). Experiments were conducted between 21° and 24°C; the temperature of the phonotaxis experiment was matched to that of the song recordings within 1.5°C. Loudspeakers were placed at adjacent corners of the arena so that the speakers and the cricket formed the corners of an equilateral triangle, 1 m on a side (Fig. 1). The sound pressure levels at the release site ranged between 65 and 70 db SPL (sound pressure level relative to 2×10^{-4} dyne/cm^2) and care was taken to equalize the sound from each speaker.

Each female was allowed 5 minutes to leave the container and an additional 5 minutes to make a choice; those that stayed in the container or that persistently walked along the edge of the arena were discarded. In order to minimize position bias the songs were alternated between the two loudspeakers at frequent intervals during the discrimination tests. Each female was tested only once, thus every choice represents a different individual.

The ability of female crickets to make species-specific phonotaxic responses in laboratory situations has been demonstrated for numerous cricket species (*9, 10*), including *Teleogryllus* (*6, 11*). In earlier experiments, hybrid T-1 females showed phonomotor maze preferences for T-1 song over either *T. oceanicus* or *T. commodus* parental songs (one-choice trials), but the females were not tested for discrimination between simultaneously playing calls (*6*), nor was T-2 tested. Our present experiment requires a two-choice discrimination (Fig. 1), and both T-1 and T-2 hybrids were tested.

When a female is placed on the floor of the arena, she takes an average of 126 seconds [standard deviation (S.D.), 81 seconds; *N*, 53 females] to walk out of her container, and another 53 seconds

Fig. 2. Female cricket photographed on the cone of a loudspeaker that is playing calling song. It is not unusual for females to climb on the speaker as part of their phonotaxic choice behavior; contact with the speaker is part of the choice criterion.

(S.D., 36 seconds; N, 204 females) to choose a speaker. Responses were usually unequivocal; paths were often direct, although somewhat zigzag, between container and speaker. A choice was defined as walking to within 1 inch (2.54 cm) of the speaker (series A), or actually touching the speaker (series B). It was not uncommon to see females actually climb upon the speaker, and sometimes even contact the speaker cone (Fig. 2). Once a female made a choice, she usually remained in the immediate vicinity of the speaker.

The F_1 hybrid females show a phonotaxic preference for the calls of their male siblings over that of males from the reciprocal cross, even though both are F_1 songs (Table 1). This is true for both F_1 cross types. The phonotaxic preference appears to be stronger for T-2 than for T-1, but the difference is not significant (*12, 13*). The *G*-test confirms sibling-specific preference at the $P < .005$ confidence level (*13*).

What are the differences between T-1 and T-2 songs that females detect for making sibling-specific discriminations? Cricket songs differ among species in carrier frequency and temporal pattern (*14*). In carrier frequency, *T. commodus* (3.5 khz) and *T. oceanicus* (4.8 khz) differ (*15, 16*). However, an earlier study of *Teleogryllus* hybrids (*15*) showed that T-1 and T-2 do not differ significantly in carrier frequency, 4.1 khz for both hybrids, and we have confirmed these results (*16*). We thus conclude that hybrid discrimination is not based on differences in carrier frequency. Certainly temporal pattern differs between parental and hybrid songs, and even between T-1 and T-2 songs, and these differences have a genetic basis (*2, 5*). Differences between the temporal patterns of T-1 and T-2 calls appear to be sex-linked (males are XO sex-determined) and are probably associated with the maternal X chromosome (*5*), although cytoplasmic factors are not excluded.

The genetic basis of sibling-specific discrimination behavior in F_1 hybrid females is unclear. While differences in the temporal pattern of T-1 and T-2 calls are apparently sex-linked, females are XX sex-determined and hence receive both maternal and paternal sex chromosomes. Thus, differences in pattern recognition by T-1 and T-2 females are not due simply to the presence of the maternal X chromosome (again, cytoplasmic factors cannot be excluded). Our results could be explained, however, by supposing that only the maternal X chromosome is expressed (selective inactivation of the paternal X chromosome) during neural development and that this differential expression influences auditory temporal pattern sensitivity at adulthood. Then both male and female siblings of a common F_1 cross type would share common (maternal) X chromosome types, and those aspects of neural function that affect pattern sensitivity (in females) and patterned motor output (in males) would be linked in both sexes. Such a mechanism would lead to the prediction that the X-linked parameters of song rhythm may be especially critical for phonoresponse. From genetic studies in vertebrates it is known that selective inactivation of sex chromosomes can occur (Lyon hypothesis) and that sometimes inactivation systematically affects the paternal X chromosome (*17*).

Sibling-specific phonotaxic responses in hybrid *Teleogryllus*, together with well-documented cases of species-specific phonotaxis in many cricket species, raise interesting questions about the neural basis of species-specific communication and its genetic control. Central pattern generators that produce the temporal characteristics of the call have been demonstrated in the nervous system of male crickets (*18*). In females, the physiological basis of sensitivity to temporal pattern has not yet been established; however, it could be achieved through neurons that function as (i) feature detectors, whose activity require particular patterns of auditory input; (ii) auditory templates, against which auditory input is compared; or (iii) combinations of both. Physiological studies in other animals indicate that species-specific feature detectors or templates may be important in auditory communication in frogs (*19*) and birds (*20*). The existence of neurons common both to central pattern generators (male) and to hypothetical feature detectors or templates (female) would help explain the coupling of male and female auditory behavior. Genetic control could be achieved by identical sets of genes acting on the same neuron types in both male and female. Thus, behavioral and genetic coupling would have a common basis. The notion that intraspecific communication is behaviorally coupled and that coupling has genetic (as well as learned, in higher animals) determinants is not novel, and has been previously proposed for crickets (*14*) and birds (*21*). The present studies provide experimental support for these ideas.

Table 1. Hybrid phonotaxic choice behavior in response to sibling and reciprocal sibling calling songs. Each column gives the total number of females that made a phototaxic choice; the data represent different individuals because no female was retested after making a choice. Series A (1973) and series B (1975) were tests run at different laboratories (*7*). Percentage of response to sibling refers to percentage of T-1 females that chose T-1 song over T-2 song, and T-2 preference for T-2 over T-1 songs. T-1 is *Teleogryllus oceanicus* ♀ × *T. commodus* ♂; and T-2 is *T. commodus* ♀ × *T. oceanicus* ♂.

Hybrid type performing discrimination	Response to T-1 song	Response to T-2 song	Response to sibling (%)
T-1			
Series A	12	1	
Series B	85	49	
Total	97	50	66
T-2			
Series A	19	66	
Series B	21	59	
Total	40	125	76

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References and Notes

1. R. D. Alexander, in *Animal Sounds and Communication*, W. E. Lanyon and W. N. Tavolga, Eds. (American Institute of Biological Sciences, Washington, D.C., 1960), pp. 38–92.
2. Y. Leroy, *C. R. Acad. Sci.* **259**, 892 (1964).
3. D. R. Bentley and R. R. Hoy, *Sci. Am.* **231**, 34 (Aug. 1974).
4. R. R. Hoy, *Am. Zool.* **14**, 1067 (1974).
5. D. R. Bentley and R. R. Hoy, *Anim. Behav.* **20**, 478 (1972).
6. R. R. Hoy and R. C. Paul, *Science* **180**, 82 (1973).
7. Site A: State University of New York, Stony Brook; site B: Cornell University, Ithaca, N.Y.
8. The tape recorders that we used were Uher 4400, Nagra IV, and Sony 350s stereo recorders. The loudspeakers were Realistic model 1197 hi-fi (30 to 17,000 hertz). We used a Uher M-514 dynamic microphone for recording cricket calls.
9. T. J. Walker, *Ann. Entomol. Soc. Am.* **50**, 626 (1957).
10. R. K. Murphey and M. D. Zaretsky, *J. Exp. Biol.* **56**, 335 (1972).

11. K. G. Hill, J. J. Loftus-Hills, D. F. Gartside, *Aust. J. Zool.* **20**, 153 (1972).
12. *G*-test (*13*); 0.1 > *P* > .05, *G* = 3.62.
13. R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969).
14. R. D. Alexander, *Evolution* **16**, 443 (1962); in *Animal Communication*, T. Sebeok, Ed. (Indiana Univ. Press, Bloomington, 1968), 167–213.
15. Y. Leroy, *Bull. Biol. Fr. Belg.* **100**, 63 (1966).
16. Unpublished results (this laboratory).
17. U. Mittwoch, *Genetics of Sex Determination* (Academic Press, New York, 1973).
18. D. R. Bentley, *Z. Vgl. Physiol.* **62**, 267 (1969).
19. L. S. Frishkopf, R. R. Capranica, M. H. Goldstein, *Proc. IEEE* **56**, 969 (1968); R. R. Capranica, in *Physiology of the Amphibia*, B. Lofts, Ed. (Academic Press, New York, in press), vol. 3.
20. P. Marler and M. Tamura, *Science* **146**, 1483 (1964); M. Konishi, *Z. Tierpsychol.* **20**, 349 (1963); *ibid.* **22**, 770 (1965); P. Marler and P. Mundinger, in *Ontogeny of Vertebrate Behavior*, H. Moltz, Ed. (Academic Press, New York, 1971), pp. 389–450.
21. P. Marler, *Soc. Res.* **40**, 293 (1973).
22. We acknowledge the help of R. Raderman, F. Ekert, V. Alones, and F. Silverberg in making discrimination tests. The comments of our colleagues R. Capranica, J. Paton, P. Narins, G. Cassady, T. Eisner, and T. J. Walker were helpful in preparing the manuscript. This work was supported by NIH grant R01 NS 11630.

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Antimetabolic Extract from the Brain of the Hibernating Ground Squirrel *Citellus tridecemlineatus*

Abstract. *Extracts of subcortical brains from hibernating ground squirrels, when injected intravenously into rats, caused a mean decrease in oxygen consumption of 35 percent and a decline in body temperature of 5°C. The effects lasted from 75 minutes to 30 hours. Brain extracts of nonhibernating squirrels caused no significant changes in these parameters.*

Lyman and Chatfield (*1*) have presented the evidence that upon entry into hibernation a reduction in metabolic rate precedes any drop in the body temperature. Keller (*2*) showed that a 40 percent reduction in basal metabolism followed ablation of certain areas of the tuberal and anterior hypothalamus in dogs. He stated "the normally elevated basal energy metabolism is a function of an endocrine hypothalamus." Subsequently, Swan and Hall (*3*) presented data that the metabolic rate of estivating lungfish in nature drops independent of a change in body temperature. This has been directly confirmed by Rienhard (*4*). Thus, evidence is accumulating that metabolic rate can be reversibly lowered in mammals and poikilotherms by mechanisms independent of the depression of biochemical reaction rates associated with decreasing temperature.

The existence of a specific endocrine factor in the torpor of hibernation was first suggested by Kroll (*5*) and Dawe and Spurrier (*6*) later showed that injections of a filtrate of blood from hibernating ground squirrels could induce onset of torpor in alert ground squirrels.

The first evidence that metabolic rate might be lowered in a homeotherm (rat) by an agent extractable from the brain of a torpid animal was that published by Swan, Jenkins, and Knox (*7*). When injected intravenously into white rats, extracts of estivating lungfish brains caused a 35 percent decline in CO_2 production followed by a decrease in body temperature of 5°C. The effect lasted only a few hours. They theorized that the extract might contain a specific antimetabolic hormone and suggested the name "antabolone" for it.

In 1972 Swan reported data derived from studies of comparative metabolism, which supported Keller's classic concept of "a *normally elevated* basal metabolism" (*8*). And that portion of the resting metabolic rate of homeotherms, which normally exists primarily to produce heat rather than energy biologically convertible to work, was suggested by Swan to be the expendable or viably depressable fraction of metabolism when accompanied by alterations in thermoregulatory mechanisms (*9*).

We now confirm that metabolic rate may be reversibly decreased in rats without a preceding drop in the level of body temperature and describe experiments in which the response of white rats to intravenous injection of an extract of the brain of hibernating ground squirrels is compared to the response following injection of a similar extract from the nonhibernating ground squirrels (*Citellus tridecemlineatus*). Oxygen consumption ($\dot{V}_{O_2}$), electrocardiograms (EKG), and body temperature (T_B) were monitored.

Ground squirrels were obtained (commercially) in October. To encourage hibernation they were housed in a quiet dark room at 6°C with adequate food and water. Most of the animals were hibernating by late November. During the next 3 months, torpid squirrels (T_B = 6° to 8°C) were killed without arousal by

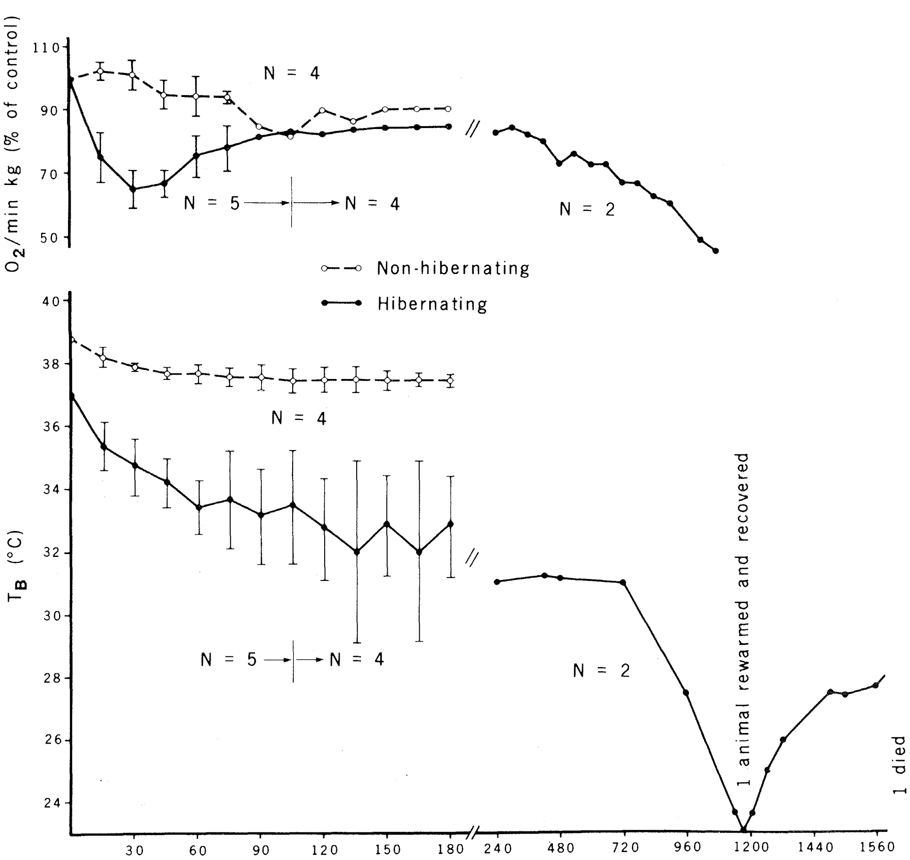

Fig. 1. Response of oxygen consumption and body temperature of white rats after intravenous injection of brain extract (300 mg/kg) from nonhibernating and hibernating ground squirrels. The ambient temperature was 24° ± 2°C.