Our results demonstrate that at least two genes, *Sh* and *Eag*, are involved in this control. It is likely that additional genes controlling the K^+ currents remain to be identified. Finally it should be noted that, although *Sh* and *Eag* affect both nerve and muscle, the K^+ currents in *Drosophila* nerve remain to be analyzed. The parameters of these currents and their alterations in the mutants may differ in detail from those in muscle.

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References and Notes

1. O. Siddiqi and S. Benzer, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3253 (1976); K. Ikeda, in *Simpler Networks and Behavior,* J. C. Fentress, Ed. (Sinauer, Sunderland, Mass., 1976), p. 140; C.-F. Wu, B. Ganetzky, L. Y. Jan, J. N. Jan, S. Benzer, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4047 (1978); C.-F. Wu and B. Ganetzky, *Nature (London)* **286**, 814 (1980).
2. J. N. Jan, L. Y. Jan, M. J. Dennis, *Proc. R. Soc. London Ser B* **198**, 87 (1977); B. Ganetzky and C.-F. Wu, *J. Neurophysiol.* **47**, 501 (1982).
3. M. A. Tanouye, A. Ferrus, S. C. Fujita, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6548 (1981).
4. L. Salkoff and R. Wyman, *Science* **212**, 461 (1981); *Nature (London)* **293**, 228 (1981).
5. J. A. Connor and C. F. Stevens, *J. Physiol. (London)* **213**, 21 (1971).
6. A. L. Hodgkin and A. F. Huxley, *ibid.* **117**, 500 (1952).
7. W. D. Kaplan and W. E. Trout III, *Genetics* **61**, 399 (1969).
8. L. Y. Jan and Y. N. Jan, *J. Physiol. (London)* **262**, 189 (1976).
9. The ventral lateral longitudinal muscle fibers of mature third instar larve (8) were used. The fibers are each identifiable and readily accessible to external medium and meet isopotential requirements (8). Ringer and methods for EJP and current clamp recordings have been described (2, 8). To eliminate Ca^{2+} current, the Ringer was modified to contain 0 mM $CaCl_2$, 0.5 mM EGTA, and 15 mM $MgCl_2$.
10. N. Suzuki and M. Kano, *J. Cell. Physiol.* **93**, 383 (1977).
11. A. Takeuchi and N. Takeuchi, *J. Physiol. (London)* **154**, 52 (1960); J. A. Connor and C. F. Stevens, *ibid.* **213**, 1 (1971).
12. To compare active currents, the linear leakage current (estimated from the I-V relations below threshold) was subtracted from the total current (Fig. 2, a to d). The cell surface area was estimated (8) from measurements with a dissecting microscope or scanning electron microscope. Calculations of membrane current density are based on the assumption of smooth surface of muscle cells and may be overestimated.
13. C.-F. Wu and F. N. Haugland, in preparation.
14. Comparison of the linear regression lines fitted to *Eag* and normal data for $V \geq 0$ mV in Fig. 3, a and b, shows that the difference in elevation or intercept is significant at the 0.005 level for I_K ($F = 8.2$, d.f. = 1, 117) and at the 0.05 level for I_A ($F = 4.9$, d.f. = 1, 85).
15. D. J. Adams, S. J. Smith, S. H. Thompson, *Annu. Rev. Neurosci.* **3**, 141 (1980).
16. We thank R. Joyner for advice on instrumentation and R. Kreber for technical help. Supported by USPHS grants NS00675, NS18500, and NS15797, and a grant from Chicago Community Trust/Searle Scholars Program to C.-F.W. and by USPHS grants NS15390 and NS00719 to B.G. Contribution No. 2560, Laboratory of Genetics, University of Wisconsin, Madison.
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Hybrid Tree Frogs: Vocalizations of Males and Selective Phonotaxis of Females

Abstract. *Male hybrids of reciprocal crosses between gray and pinewoods tree frogs* (Hyla chrysoscelis × H. femoralis) *that were raised to sexual maturity in the laboratory produced distinctive vocalizations. Hybrid females preferred the calls of hybrids to calls of gray tree frogs and also chose synthetic calls with a pulse repetition rate typical of the hybrids in preference to calls with a rate typical of pinewoods tree frogs.*

Information about the extent to which sound production and recognition are matched or coordinated in interspecific hybrids may help to elucidate the mechanisms and evolution of animal communication. Most studies of the acoustic behavior of F_1 hybrids have dealt with the analysis of sound production (1), and data on the selective responsiveness of hybrids to acoustic signals have been available only for insects (2, 3). We report that female hybrids between two species of tree frogs preferred the calls of male hybrids to those of one of the parental species. Females also discriminated between synthetic calls that differed in a temporal property thought to be critical for signal recognition; they preferred a stimulus typical of calls produced by hybrids.

Reciprocal hybrids between the gray tree frog (*Hyla chrysoscelis*) and the pinewoods tree frog (*H. femoralis*) were obtained by switching the males of mating pairs before oviposition began. The rates of fertilization and survival to sexual maturity were high, and males first began calling 7.5 months later. Sounds of male hybrids were recorded in a semianechoic, temperature-regulated chamber; selective phonotaxis of female hybrids was tested in two-speaker playback experiments conducted at 23° to 25°C in the same chamber (4).

Vocalizations of the parental species have been characterized (5). Males of *H. chrysoscelis* produce discrete trains of sound pulses. The pulse repetition rate is stereotyped and averages 44 sec^{-1} in populations in eastern Georgia at about 24°C. Males of *H. femoralis* produce pulses in an irregular, often continuous fashion for several minutes; the pulse repetition rate varies between 6 and 12
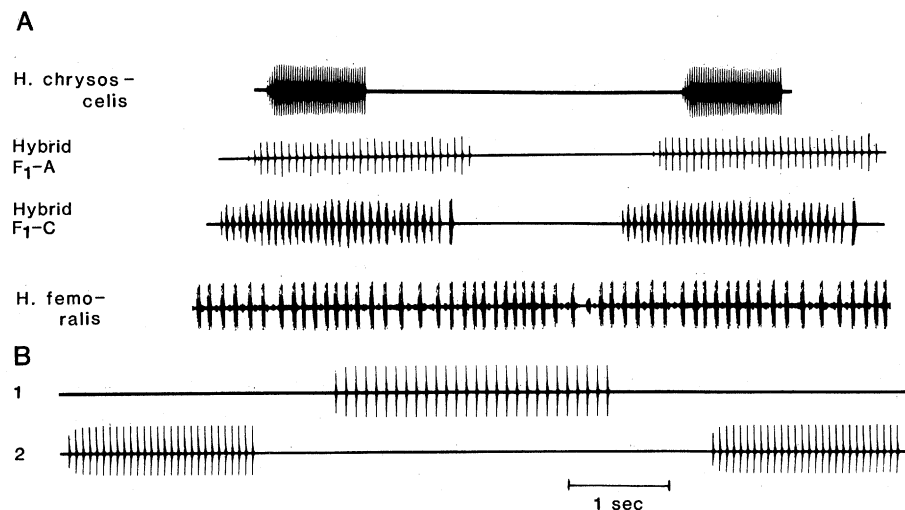

A

H. chrysos-
   celis

Hybrid
F_1-A

Hybrid
F_1-C

H. femo-
   ralis

B

1

2

├─────┤
1 sec

Fig. 1. (A) Oscillograms of representative vocalizations of *Hyla chrysoscelis, H. femoralis,* and F_1 hybrids recorded at 23° to 25°C. These calls were used in playback experiments with female hybrids. The calls of the parental species were recorded (tape recorder, Nagra or Uher; microphone, Sony C-22 or Sennheiser 815) in eastern Georgia, the region from which the parents of the hybrids were collected. Hybrid calls were categorized into three types. F_1-A calls had shorter pulses than F_1-C calls; F_1-B calls (not illustrated) had both short and long pulses (4). Only part of the vocalization of *H. femoralis* is shown; males of this species often call more or less continuously; *H. chrysoscelis* and hybrids, by contrast, organize pulses into discrete trains such as those shown. (B) Oscillograms of synthetic calls modeled after the F_1-A hybrid call. The two acoustic stimuli differed only in pulse repetition rate, duty cycle (ratio of pulse duration to pulse period), and call duration. The pulse repetition rates were chosen to approximate the mean rates of *H. femoralis* (oscillogram 1, 8 sec^{-1}) and F_1 hybrids (oscillogram 2, 15 sec^{-1}) at 24° to 25°C. The timing of the two calls during presentation to female hybrids was as shown. Each was recorded on and played back from a separate track of a stereophonic recorder (Nagra IV-S). When the "fast" root-mean-square sound pressure level (SPL) of the two stimuli was equalized, the discrepancy in their peak SPL was only 2 decibels.

sec^{-1} (mean, 8 sec^{-1}) at 24°C. Pulses produced by *H. femoralis* are longer and more complex than those of *H. chrysoscelis*. Although the harmonic structures of the two parental calls differ, the band containing the most sound energy (the dominant frequency band) is centered at about the same frequency in the calls of the two species.

The vocalizations of F_1 hybrids of the two reciprocal crosses were indistinguishable. Some acoustic properties were similar to one or the other of the parental species, and others were intermediate (Fig. 1A) (6). As in *H. chrysoscelis*, male hybrids organized pulses into discrete trains. Pulse repetition rate, the most stereotyped temporal property (6), averaged about 15 sec^{-1} at 24° to 25°C; in this respect the vocalizations of hybrids were more like the calls of *H. femoralis* than those of *H. chrysoscelis*. The dominant frequency bands of calls made by hybrids were similar to those of the parental forms (7).

The phonotactic behavior and preferences of females of the reciprocal crosses were similar. Females responded by moving toward and sometimes touching a loudspeaker. Given a choice between the calls of *H. chrysoscelis* and those of hybrids (types F_1-A and F_1-C in Fig. 1), females preferred the calls of hybrids (Fig. 2). Females did not show a preference between the calls of hybrids and those of *H. femoralis* (Fig. 2).

The absence of a preference for the calls of hybrids to the calls of *H. femoralis* might be attributable to two factors. First, the pulse repetition rate of the *H. femoralis* stimulus was highly variable and sometimes matched that of the hybrid calls. Second, the pulses of the *H. femoralis* call were longer and were produced more or less continuously; the total amount of acoustic stimulation produced by this call was thus much greater over long test sessions than that produced by the hybrid call (Fig. 1A). These variables were eliminated or minimized by synthesizing the signals depicted by the oscillograms of Fig. 1B. The pulse repetition rates of these artificial calls had values corresponding to the means in the vocalizations of F_1 hybrids and *H. femoralis* at 24° to 25°C, that is, 15 sec^{-1} and 8 sec^{-1}, respectively. Female hybrids clearly preferred the 15 sec^{-1} call to the 8 sec^{-1} call. Experiments with females of *H. chrysoscelis* and its sibling, *H. versicolor*, indicate that pulse repetition rate is the most important property for call recognition (8).

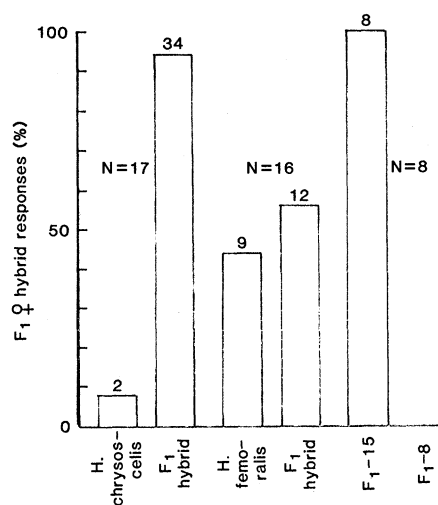Some properties of a signal produced by an animal must constitute a pattern that can be identified by another animal

Fig. 2. Summary of phonotactic responses of F_1 females in two-stimulus playback experiments at 23° to 25°C (4). The number of responses is indicated above each bar of the histograms (*N*, number of females responding). Females of the two reciprocal crosses responded in the same fashion, and the results with the F_1-A and F_1-C call types were the same. If only the first response of each female is considered, the results were as follows: F_1 hybrid versus *H. chrysoscelis*, 20:1 ($P < .001$); F_1 hybrid versus *H. femoralis*, 11:9 ($P > .5$); and F_1 at 15 sec^{-1} versus F_1 at 8 sec^{-1}, 8:0 ($P < .01$). *P* values are results of two-tailed binomial tests of the null hypothesis of no preference.

of the same species. The two individuals communicate by means of a common code, even if one sex never produces signals but responds to the signals of the other sex. In discussing acoustic insects, Alexander (9) suggested that the two sexes might share a neural network in the central nervous system. Regulated by the same genes, the network would pattern the motor output of the male and could serve as a reference or template for the processing of afferent activity in the auditory system of the female. The evolution of the communication system thus would be simplified because any mutation affecting the network would simultaneously modify both sender and receiver mechanisms in the same way (10). A common code can arise even if the sender and receiver mechanisms are regulated by few or none of the same genes. Only males producing signals with "correct" properties will attract appropriate mates; similarly, only females that respond to signals with the correct properties will find an appropriate mate.

Hoy *et al.* (2) showed that female hybrids between two species of Australian field crickets not only responded selectively to the songs of male hybrids rather than to those of the parental species, but also preferred those of the same

reciprocal cross. These authors suggested that the sender and receiver mechanisms in these animals are "genetically coupled." By contrast, Helversen and Helversen (3) found that hybrids between two species of European grasshoppers produced highly variable songs and that female hybrids usually displayed little, if any, response specificity with respect to hybrid and parental songs. As Elsner and Popov (11) and Gould (12) point out, genetic coupling is probably not a general phenomenon.

Female hybrid tree frogs were partially selective with respect to natural vocalizations and probably completely selective with respect to pulse repetition rate. Although neither our results nor those of Hoy and his colleagues constitute unequivocal evidence for the genetic coupling hypothesis, the behavior of the hybrid crickets and tree frogs is consistent with the mechanism proposed by Alexander (9). The specificity of hybrids demonstrated by behavioral studies warrants the search for common neural elements in the sound production and recognition systems of these animals.

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References and Notes

1. A. S. Ewing, *Anim. Behav.* **17**, 555 (1969); T. S. McGrath, M. D. Shalter, W. M. Schleidt, P. Sarvella, *Nature (London)* **237**, 47 (1972); H. Schneider and H. Eichelberg, *Oecologia (Berlin)* **16**, 61 (1974).
2. R. R. Hoy and R. C. Paul, *Science* **180**, 82 (1973); R. R. Hoy, J. Hahn, R. C. Paul, *ibid.* **195**, 82 (1977).
3. D. von Helversen and O. von Helversen, *J. Comp. Physiol.* **104**, 273 and 301 (1975).
4. J. Doherty, thesis, University of Missouri, Columbia (1982). Calls of hybrids were recorded by a sound-activated recording system (Stellavox Sp 7 recorder; Sennheiser 416 microphone). Some frogs called spontaneously; others were induced to call by hormone injections and playbacks of previously recorded hybrid calls. There is no evidence that acoustic experience affects the development of mating calls in *H. chrysoscelis* [J. Burger, thesis, University of Missouri, Columbia (1980)]. Each female hybrid was placed midway between two speakers (Analog-Digital-Systems 200), 2 m apart; the frog was initially positioned with the snout-vent axis perpendicular to the speaker axis. Playback of the experimental stimuli by a stereophonic recorder (Nagra S) (one kind of sound per channel) began 15 to 30 seconds after placement of the female; the sound pressure level of the two stimuli was equalized at 75 decibels (relative to 20 μN/m²) root-mean-square. A response was tabulated when a female moved to within 30 cm of a speaker.
5. H. C. Gerhardt, thesis, University of Texas, Austin (1970); *Behaviour* **49**, 130 (1974).
6. Call and pulse durations were slightly longer in the calls of hybrids than in the calls of *H. chrysoscelis*. The mean coefficient of variation of the pulse repetition rate in hybrids was 14.5 percent (range, 5 to 22 percent) as compared with 4 percent (range, 3 to 4 percent) in *H. chrysoscelis* and 29 percent (range 8 to 79 percent) in *H. femoralis*.
7. Dominant frequency bands were at 1.1 to 1.6 kHz and at 2.0 to 3.0 kHz in *H. chrysoscelis* and in hybrids, and at 1.7 to 2.6 kHz in *H. femoralis* (4). Our selection of the parental species was guided in part by the fact that their calls have about the same dominant frequency. The pe-

ripheral auditory system of anurans is roughly "tuned" to the energy in the mating call [R. R. Capranica, in *Frog Neurobiology*, R. Llinas and W. Precht, Eds. (Springer-Verlag, New York, 1976), p. 551], and we wanted to be confident that any response specificity we might find in hybrids would be based mainly on temporal properties of vocalizations and not strongly biased by "peripheral filtering." Behavioral coupling might occur in hybrids between species of different size for the trivial reason that both call frequency and frequency sensitivity could be correlated with size, and hybrids would be intermediate in this respect.

8. H. C. Gerhardt, *Science* **199**, 992 (1978); *Am. Zool.* **22**, 581 (1982).
9. R. D. Alexander, *Evolution* **16**, 443 (1962).
10. One potential theoretical problem with Alexander's proposal is that brothers and sisters would be expected to mate with each other preferentially. If the genetic load is high (many homozygous recessive lethals), the negative effects of inbreeding could, at least initially, counterbalance the advantages of such a simple evolutionary mechanism.
11. N. Elsner and A. V. Popov, *Adv. Insect Physiol.* **13**, 229 (1978).
12. J. L. Gould, *Ethology: The Mechanisms and Evolution of Behavior* (Norton, New York, 1982), pp. 298–307.
13. We thank C. Baysinger, J. Risch, and R. Daniel for technical and field assistance. Supported by grants from the National Science Foundation, National Institutes of Health, and University of Missouri Research Council (to H.C.G.).
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Erectile Mechanisms in Man

Abstract. *Increases in penile circumference during sleep-related erections in human subjects closely reflected increases in penile blood flow, and bursts of activity in the bulbocavernosus and ischiocavernosus muscles were temporally related to these increases. The penile arterial system and the perineal muscles appear to have important coordinated roles in human penile erection. Monitoring sleep-related erections and penile blood flow holds promise for the study of erectile mechanisms and dysfunction and for screening of drugs.*

Human penile erection is a complex psychophysiological phenomenon dependent on multiple systems, but hemodynamic processes produce the major proximal changes necessary for the initiation and maintenance of erection. The nature of these changes has been postulated to involve restricted venous outflow, both increased arterial inflow and restricted venous outflow, or increased arterial inflow (*1*). Early research on this problem relied on anatomical examinations (*2*). In more recent studies cavernosography and phalloarteriography have been used as attention was directed to the dynamics of the process (*3*). The controversy over the relative contributions of the arterial and venous systems nevertheless remains unresolved.

Few investigators in this area ascribe any role in human erection to the perineal muscles. The studies of Bors and Comarr (*4*) and Kollberg *et al.* (*5*) are typically cited as evidence negating such a role. In the former study, patients were diagnosed as having lower motor neuron lesions of sacral segments on the basis of an absent bulbocavernosus reflex and abnormal detrusor muscle function. None of these patients experienced reflex erections, but some had psychogenic erections. Absence of the bulbocavernosus reflex was determined by manual examination (*6*), not by electrophysiological evaluation, and data on erectile function came from patient interviews, not from direct observation. Kollberg *et al.* did an electromyographic study of ejaculation achieved by masturbation. They did not monitor degree of erection. In summarizing some observations made from the start of erection, the authors stated that activity in the striated muscles of the pelvic floor sometimes increased before and during erection. They concluded that this activity is not necessary for the occurrence of erection, but noted that its importance in erection remains unclear.

Our previous observations suggest that the bulbocavernosus and ischiocavernosus muscles do play a role in human erection (*7*). Bursts of activity in the muscles were seen to precede slightly and accompany the penile pulsations characteristic of early phases of sleep-related erections. Penile circumference was usually greater after a pulsation than before. We speculated that contraction of the muscles may sporadically pump blood into the penis to assist in the initiation and maintenance of erection.

Failure to achieve erection is a common complaint, especially among older men. As the U.S. population grows older, the complaint will become even more widespread. The more we learn about the mechanisms of erection, the better will be the medical care available to new victims. Several methodological improvements may pave the way for a clearer understanding of both the hemodynamic and neuromuscular mechanisms. To our knowledge, in all studies to date erections have been induced by purely artificial means (saline infusion into the corpora cavernosa) or by presentation of erotic pictures. In no study has detailed simultaneous examination of penile erection and penile hemodynamics been made by noninvasive measurement of segmental pulsative blood flow. Finally, no study has combined examination of penile blood flow with monitoring of perineal muscle activity.

We examined penile blood flow and its relation to bulbocavernosus and ischiocavernosus muscle activity (BCA) during nocturnal penile tumescence (NPT) episodes in seven healthy, potent men 22 to 30 years of age. NPT occurs naturally and regularly during sleep in all healthy boys and men; most of the time it is temporally related to rapid-eye-move-
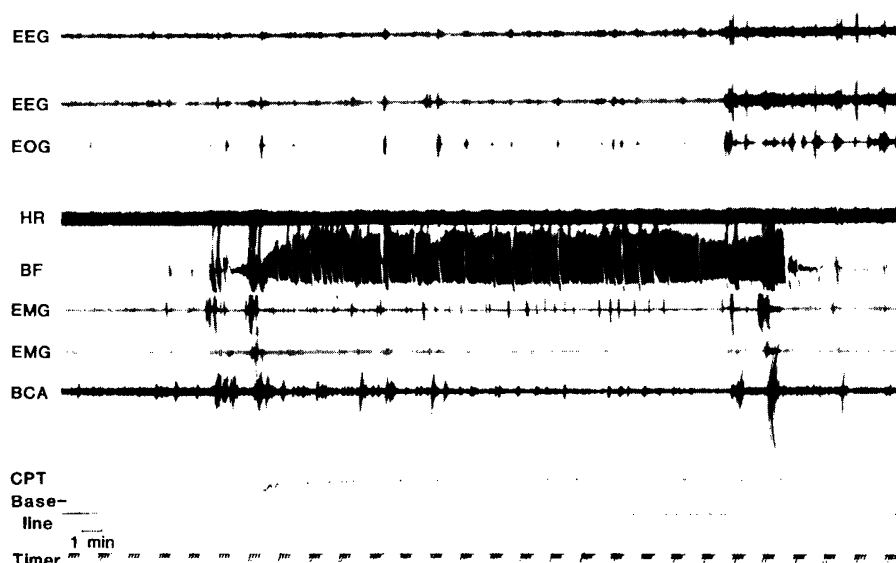

Fig. 1. Polygraph tracing (0.25 mm/sec) for a representative NPT episode. Channels are electroencephalograph (*EEG*), electrooculograph (*EOG*), heart rate (*HR*), blood flow (*BF*), left- and right-leg electromyograph (*EMG*), bulbocavernosus and ischiocavernosus muscle activity (*BCA*), circumference change at the penile tip (*CPT*), penile tip circumference baseline, and timer.