

in favor of the proximal orientation. The energy difference required to explain the observed difference in binding affinity can be calculated from the Boltzmann distribution law (13). At 37°C a 4 to 1 preponderance of the proximal 3' iodine orientation, corresponding to the relative ratios of free to bound  $T_3$  and  $T_4$  in purified TBG solution, would be maintained by an energy difference of 854 calories. Using molecular orbital theory, Camerman and Camerman calculated a much higher energy difference (132 kcal), but they considered this likely to be an overestimation (2). The lower energy difference, which is consistent with the present hypothesis, would seem more likely for the coordination of the 3' iodine with the  $\pi$  electron system of the phenylalanine ring, which is the probable basis for the predominance of the proximal 3' iodine orientation (14). Kier and Hoyland (15), also using molecular orbital theory, calculated only a small energy difference that they consider to be insignificant. It is hoped that a more direct estimate of the energy difference between distal and proximal 3' iodine orientation will be available shortly from nuclear magnetic resonance spectroscopy.

GEORGE C. SCHUSSLER

Department of Medicine, State University of New York, Buffalo 14203

#### References and Notes

- E. C. Jorgensen, *Proc. Mayo Clinic* **39**, 560 (1964).
- N. Camerman and A. Camerman, *Science* **175**, 764 (1972).
- J. Robbins and J. E. Rall, *J. Clin. Invest.* **34**, 1331 (1955).
- E. C. Jorgensen and P. N. Kaul, *Amer. J. Pharm.* **48**, 653 (1959); N. Zenker and E. C. Jorgensen, *J. Amer. Chem. Soc.* **81**, 4643 (1959).
- E. C. Jorgensen, P. A. Lehman, C. Greenberg, N. Zenker, *J. Biol. Chem.* **237**, 3832 (1962).
- G. C. Schussler and J. E. Plager, *J. Clin. Endocrinol.* **27**, 242 (1967).
- K. Sterling, P. Rosen, M. Tabachnick, *J. Clin. Invest.* **41**, 1021 (1962).
- S. H. Ingbar and N. Freinkel, *Recent Progr. Horm. Res.* **16**, 353 (1960).
- J. J. Pensky and J. S. Marshall, *Arch. Biochem. Biophys.* **135**, 304 (1969).
- Polyacrylamide gel electrophoresis of rat serum at pH 9.0 shows 18 percent of  $T_4$  associated with a protein cathodal to albumin and 55 percent associated with a prealbumin, the remainder binding to albumin [P. J. Davis, S. W. Spaulding, R. I. Gregerman, *Endocrinology* **87**, 978 (1970)]. However, at pH 7.4 in polyacrylamide gel,  $T_4$  is bound only to prealbumin, and  $T_3$  to albumin. The same electrophoretic system demonstrates TBG binding of  $T_3$  and  $T_4$  in human serum (G. C. Schussler, unpublished observations).
- J. H. Oppenheimer and R. R. Tavernetti, *J. Clin. Invest.* **41**, 2213 (1962); G. C. Schussler, *J. Pharmacol. Exp. Ther.* **178**, 204 (1971).
- Conformational difference is not the only possible basis for the difference in binding affinity for  $T_3$  and  $T_4$ . Sterling and Tabachnick [*J. Biol. Chem.* **236**, 2241 (1961)] suggest that greater ionization of the  $T_4$  phenolic hydroxyl at pH 7.4 may account for tighter binding to albumin and possibly to TBG as well.
- According to the Boltzmann distribution law the molar ratio of the two conformations of triiodothyronine will be
 
$$\frac{N(d)}{N(p)} = e^{-[E(d)-E(p)]/RT}$$
 where d and p refer to the distal and proximal orientations of the 3' iodine,  $N$  is the molar concentration of each such conformation,  $E$  is the total molecular energy of each conformation in calories,  $R$  is the gas constant (1.9869 cal per degree C per mole), and  $T$  is the absolute temperature.
- T. M. Cromwell and R. L. Scott, *J. Amer. Chem. Soc.* **72**, 3825 (1950); R. M. Keefer and L. J. Andrews, *ibid.* **77**, 2164 (1955).
- L. B. Kier and J. R. Hoyland, *J. Med. Chem.* **13**, 1182 (1970).
- I thank E. C. Jorgensen for providing the triiodothyronine analogs, J. S. Marshall for a gift of purified human TBG, and D. A. Fisher, R. J. Kurland, D. Chandler, and N. Camerman for helpful discussions; J. Pfohl and R. Honour provided technical help. Supported by PHS grant 5-R01-AM11454.

4 April 1972; revised 14 July 1972

## Wing Movements of Calling Katydid: Fiddling Finesse

**Abstract.** *Stridulating Uhler's katydids produce the most complex song known for insects. Series of four types of sounds are made in stereotyped sequence. Sound-synchronized high-speed photography reveals that each type of sound is produced by a distinctively different wing-movement cycle. The most complex of these cycles includes a two-step closure and a nearly silent close-open movement.*

The species-specific calling songs of male crickets and katydids (Orthoptera: Gryllidae and Tettigoniidae) have been used in studies of systematics (1), communication (2), biophysics (3), and neurophysiology (4). Such sounds are made by the scraper of one fore wing stroking the file of the other. Most species use only one type of wing-movement cycle during calling, and hence produce only a single "phonatome," that is, a single major acoustical unit corresponding to a cycle of wing movement (5). Their songs, then, are sequences of a single phonatome. On the other hand, some species use two or more stroking techniques during calling, and hence their songs include sequences of two or more phonatomes.

Although earlier workers have used photography to determine the relation between wing movements and the sounds produced during calling, in each

case the sounds investigated suggested repetitions of a single phonatome, and a single type of wing-movement cycle was indeed found (6, 7). This report describes the relation between wing movements and sounds in Uhler's katydid, the only insect known to produce four phonatomes (8).

These katydids produce sequences of each of their four phonatomes in a stereotyped pattern lasting 8 seconds or longer. In the species studied here the calling song (Fig. 1A) begins with a sequence of 50 to 80 phonatomes of one type (hereafter designated type I). Immediately following is a sequence of 7 to 12 type II phonatomes and a sequence of 4 to 7 type III phonatomes. After a brief pause the katydid produces another sequence of 4 to 7 type III phonatomes. It may produce additional sequences of 4 to 7 type III phonatomes, but eventually it interposes one

Table 1. Comparison of four types of phonatomes produced by Uhler's katydids from Washington County, Ohio.

Phonatome type	Usual number of phonatomes in sequence	Approximate duration at 25°C (msec)	Approximate repetition rate at 25°C (sec <sup>-1</sup> )	Maximum sound intensity (relative)*	Amplitude of wing opening (relative)	Special features
I	50-80	70	14	0-20	50	Intensity increases during sequence; short pause during closing
II	7-12	110	9	22-26	100	Long pause during closing; nearly silent close-open
III	4-7†	45	23	28-0	100-65	Intensity decreases during sequence; wing-opening amplitude decreases during sequence
IV	3-14	20	2	18-22	20	Wing movements slight

\*Based on decibel readings from a tape recorder volume-unit meter (indicates the intensity of the volume of the signal during the tape recording); agrees with subjective impression of intensity. Frequencies above 20 kHz were excluded by the microphone. †More than one sequence of 4 to 7 type III phonatomes may be produced without intervening sounds.

or more sequences of 3 to 14 type IV phonatomes. The katydid pauses before beginning a new bout of calling with 50 to 80 type I phonatomes. In the sequences of type I phonatomes the intensity gradually increases, and in the sequences of type III phonatomes the intensity rapidly decreases (9).

By a previously described technique of high-speed sound-synchronized photography (7), we filmed five calling males at 1000 to 2000 frames per second. We simultaneously tape-recorded the calls. Frame-by-frame measurement of wing position and filmed oscillographic trace revealed that the four phonatomes were produced by the wing-stroking movements illustrated in Fig. 1, B-E, and described below and in Table 1.

The type I phonatome (Fig. 1, B and C; two individuals filmed;  $n = 23$  wing-movement cycles analyzed) is produced by a rapid opening of the fore wings followed by a slow closing. The closing usually has a hesitation near the end that produces a break in the sound longer than that occurring at either reversal of the direction of wing movement. The slow increase in intensity during a sequence of type I phonatomes is apparently produced by increased force of contact and not by change in the amplitude of wing movement.

During the type II phonatome (Fig.

1, C and D; three individuals;  $n = 18$ ) the wings are opened more widely and closed more completely than in the type I phonatomes. The closing movements are faster, and the pause between the first and second portions of closing lasts longer than the closing movements themselves. The sound produced by the second portion of closing begins abruptly, suggesting release of energy mechanically stored during the pause. Opening immediately follows the second portion of closing and produces a burst of sound similar to that of the corresponding movement in type I phonatomes. After this acoustically effective opening,

the wings partially close and then open more fully. This close-open movement is nearly silent.

The type III phonatome (Fig. 1, C and D; four individuals;  $n = 22$ ) involves a rapid closing followed by a rapid opening. The extent of opening decreases during a sequence, yet the duration of closing increases. These two changes have the effect of maintaining a fairly constant period for successive type III phonatomes except for the terminal one or two.

The type IV phonatome (Fig. 1E; one individual;  $n = 5$ ) is a two-pulse, tick-like sound made by scarcely perceptible

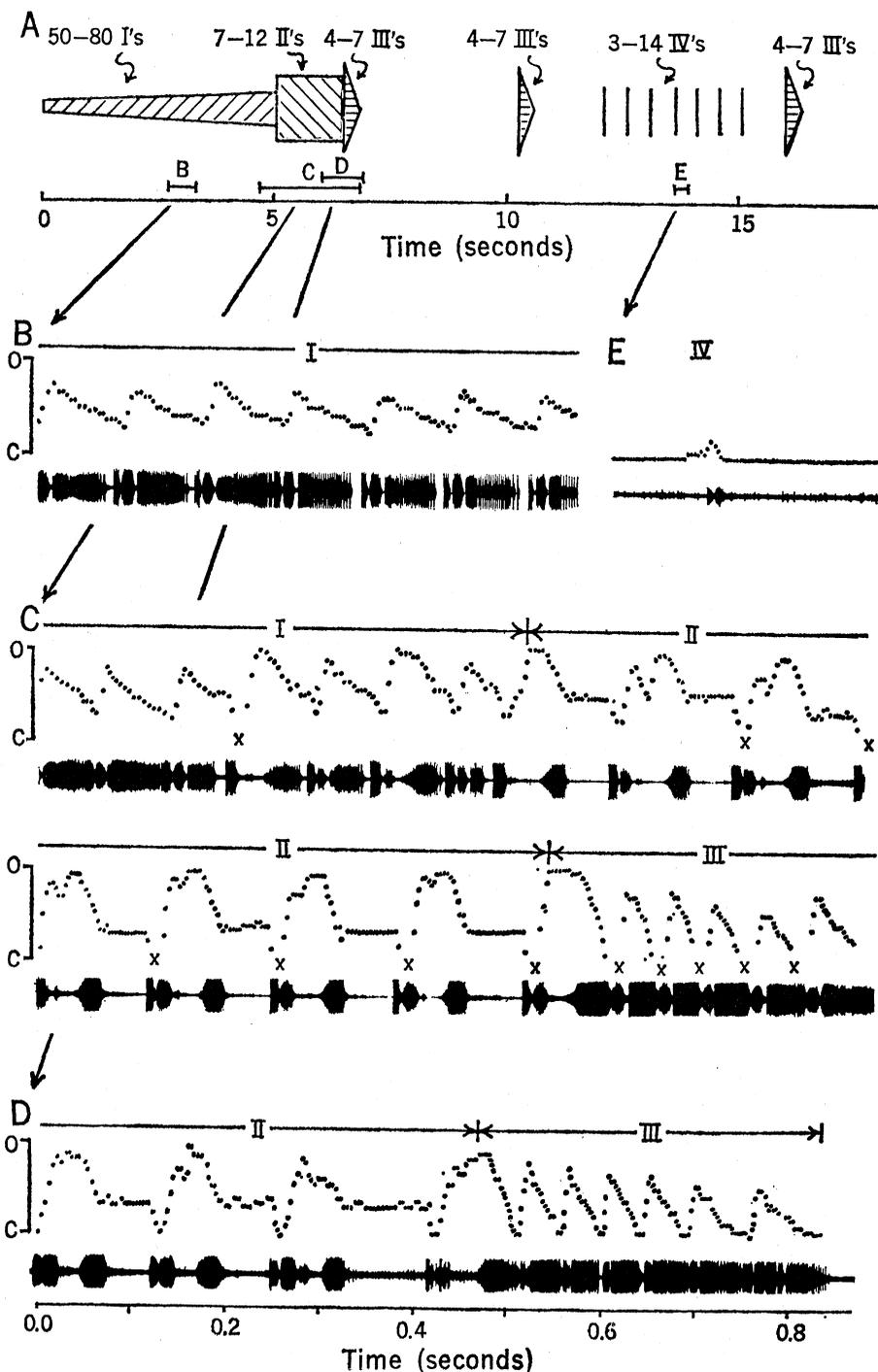


Fig. 1. Calling song and corresponding wing movements of Uhler's katydid. (A) Usual pattern of phonatome production during calling song (see text). The vertical dimension indicates relative intensity. Horizontal lines just above the time scale indicate sources of details B-E. (B-E) Wing movements (dots) and corresponding oscillograms. Wing movement is shown by the extent of wing separation measured during every fifth frame of film (on the vertical scale at the left O, fully open; C, fully closed). Roman numerals above each graph indicate the type of phonatome being produced. Each wing-movement graph is from a different film, and only (B) and (D) are of the same individual. The amplitudes of the various oscillograms are not to the same scale. Frequencies below 10 kHz [below 20 kHz for (E)] were filtered out before the oscillogram was made. In (E) the camera noise is still conspicuous. (B) Graph of 6½ type I phonatomes; the briefest spikes probably correspond to the striking of individual file teeth. (C) (Graph continues from one line to the next) Graph of 7 type I phonatomes (final 5 foretelling transition to type II), 7 type II phonatomes, and 6 type III phonatomes (x's indicate frames for which the extent of closure could not be measured). (D) Graph of 3 type II, and 7 type III phonatomes. (E) Graph of 1 type IV phonatome.

wing movements. From our film we could not positively associate a particular wing movement with either of the two pulses. Twenty independent analyses of the five filmed type IV phonotomes showed these apparent movements during the first pulse: opening,  $n = 7$ ; closing,  $n = 1$ ; no movement,  $n = 12$ . Corresponding figures for apparent movements during the second pulse were as follows: opening,  $n = 2$ ; closing,  $n = 14$ ; no movement,  $n = 4$ . The first pulse is probably produced by an opening movement and the second by a closing.

The transitions from type I to type II and from type II to type III phonotomes are distinctive. The sequence of type I phonotomes ends with several that are variable (Fig. 1C; two films; two individuals). The amplitude of wing movement is greater (suggesting type II), the period becomes shorter (suggesting type III), and sometimes opening or a portion of closing is silent. Sometimes a group of such erratic type I phonotomes is followed by a short sequence of regular ones, that gives way again to erratic type I and then to type II phonotomes. Perhaps the katydid successfully shifts to type II phonotomes after an initial failure. The transition from type II to type III phonotomes is sudden (Fig. 1, C and D; four films; four individuals). The final type II phonotome has a slightly prolonged hold during closing, and the initial type III phonotome has a closing sound that begins gradually (as in type II phonotomes).

The complexity of wing movements in this species far exceeds any previously described. Those working with simple movements in one- or two-phonotome species (2-4) should note the challenges that remain. Systematists should note that wing-movement cycles provide an important new clue to homology and analogy among signals (10). Those taxonomists who have assumed that the distinctive features of the calling song can be deduced from features of the stridulatory apparatus (11) should note that four phonotomes come from a single apparatus and that the variety of distinctive calling songs one such apparatus might produce by changes in the sequence and timing of the four phonotomes would easily exceed the number of species of katydids.

The structure of the stridulatory file of Uhler's katydid merits comment. For instance, one might expect that the file would show some specialization facilitating the two-step closures of type I and type II phonotomes. In fact, certain

species of Phaneropterinae (the subfamily including Uhler's katydid) have the most complex files known: some files have sharp bends, and others have sudden transitions in the structure and spacing of the teeth (12). However, the file of Uhler's katydid is remarkably ordinary—a row of nearly uniform teeth gently curving at either end (see cover). The calling songs of phaneropterines with complex files are unknown.

THOMAS J. WALKER, DONALD DEW  
*Department of Entomology and  
 Nematology and Communication  
 Sciences Laboratory, Department of  
 Speech, University of  
 Florida, Gainesville 32601*

#### References and Notes

1. See, for example: R. D. Alexander, *Evolution* 16, 443 (1962); D. C. Rentz and J. D. Birchim, *Mem. Pacific Coast Entomol. Soc.* 3, 169 (1968); T. J. Walker, *Ann. Entomol. Soc. Amer.* 62, 945 (1969).
2. See, for example: B. Dumortier, *Ann. Epiphyt. Paris* 14, 5 (1963); J. D. Spooner, *Anim. Behav.* 16, 197 (1968); R. D. Alexander, in *Animal Communication*, T. A. Sebeok, Ed. (Indiana Univ. Press, Bloomington, 1968), p. 167.
3. See, for example: H. C. Bennet-Clark, *J. Exp. Biol.* 52, 619 (1970); *Nature* 234, 255 (1971); W. J. Bailey and W. B. Broughton, *J. Exp. Biol.* 52, 507 (1970); G. K. Morris, *Can. Entomol.* 102, 363 (1970).
4. See, for example: W. Kutsch and F. Huber, *Z. Vergh. Physiol.* 67, 140 (1970); D. R. Bentley and R. R. Hoy, *Science* 170, 1409 (1970); D. R. Bentley, *ibid.* 174, 1139 (1971); D. Möss, *Z. Vergh. Physiol.* 73, 53 (1971); R. K. Josephson and R. C. Halverson, *Biol. Bull.* 141, 411 (1971).
5. Y. Leroy [Signaux Acoustiques, Comportement et Systématique de Quelques Espèces de Gryllides (Orthoptères, Ensifères) (Fanlac, Périgneux, France, 1966), p. 16] uses phonotome in this sense. The terms "chirp" and "syllable" have also been used but have other widely recognized acoustical meanings.
6. G. W. Pierce, *The Songs of Insects* (Harvard Univ. Press, Cambridge, Mass., 1948); F. Pasquinely and M.-C. Busnel, in *Colloque sur l'Acoustique des Orthoptères*, R.-G. Busnel, Ed. (Institut National de Recherche Agronomique, Paris, 1954), p. 146; E. S. Thomas and R. D. Alexander, *Occas. Pap. Mus. Zool. Univ. Mich.* 626 (1962), p. 26; W. J. Davis, *Anim. Behav.* 16, 72 (1968).
7. T. J. Walker, J. F. Brandt, D. Dew, *Ann. Entomol. Soc. Amer.* 63, 910 (1970).
8. Uhler's katydid constitutes a complex of at least five sibling species. All are now known as *Amblycorypha uhleri* Stal and all have calling songs with four phonotomes. The specimens photographed in this study were from Washington County, Ohio, where only one of the siblings occurs.
9. The song of this species of Uhler's katydid is more fully illustrated by R. D. Alexander, in *Animal Sound and Communication*, W. B. Lanyon and W. N. Tavolga, Eds. (American Institute of Biological Sciences, Washington, D.C., 1960), p. 72. The adaptive significance of the four phonotomes is not known, but three of them may be homologous to the three of the Texas bush katydid, for which J. D. Spooner has demonstrated distinctive functions [*Anim. Behav.* 12, 235 (1964)].
10. Distinctive wing-movement cycles reveal distinctive neuromuscular mechanisms that would otherwise remain hidden to the systematist. These underlying mechanisms should be less subject to rapid evolutionary change than the sound patterns themselves, since only the sound patterns function directly in reproductive isolation.
11. W. W. Moss, D. A. Nickle, M. G. Emsley, *Notulae Natur. Philadelphia* 432, 8 (1970).
12. Y. Leroy, *C. R. H. Acad. Sci. Paris* 270, 96 (1970); M. G. Emsley and D. A. Nickle, *Proc. Acad. Natur. Sci. Philadelphia* 12, 25 (1969).
13. We thank J. J. Whitesell and J. C. Webb for technical assistance and Dr. J. E. Lloyd for helpful suggestions and for criticizing the manuscript. Supported by NSF grant GB 20749 and NIH grant NB 06459. Florida Agricultural Experiment Stations journal series No. 4356.

27 March 1972

## Galactokinase: Evidence for a New Racial Polymorphism

**Abstract.** *Activities of galactokinase and galactose-1-phosphate uridylyltransferase in red cells were assayed in a mixed racial population of 645 pregnant women. The distribution of individual transferase activities for black subjects was the same as that for whites. In contrast, the distributions of individual galactokinase activities differed significantly in blacks and whites, the mean for the black population being 30 percent lower than the mean for the white population. The same racial difference was found when red cell galactokinase activity was examined in males and in newborns. Because low-, intermediate-, and high-galactokinase activities appear to segregate within several black families, this observed difference suggests a new, racially determined enzyme polymorphism.*

Galactokinase and galactose-1-phosphate uridylyltransferase are essential for the utilization of galactose in man. Deficiency of each of these enzymes is associated with an autosomal recessive inborn error in metabolism known to cause a clinically significant disorder (1). Galactokinase deficiency is characterized by juvenile cataracts. Transferase deficiency, or "classical" galactosemia, is associated by hepatosplenomegaly, cataracts, and failure to thrive. Heterozygotes for either of these

enzyme deficiency diseases were once thought to be easily distinguished by quantitative assay of red cell enzyme activity, because they have half the normal enzyme activity. The identification of the Duarte variant of human transferase (2), an allele that produces about half the activity of the wild type allele, made it necessary to supplement quantitative enzyme data with appropriate family studies or electrophoresis or both techniques (3) in order to establish transferase genotypes. The results