other strains (*6*), suggesting that albinism may influence not only escape from water but other forms of behavior as well. Previous investigations have revealed an inability of the A strain to learn a running response in a test of avoidance of electric shock, and a tendency toward low activity (freezing) and a high rate of emotional defecation in a stressful or aversive situation (*7*); our water escape test is also a measure of an active response to aversive stimulation. All these findings together suggest that albinism may be associated in a general way with activity, or with responsiveness to aversive stimuli.

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Courtship Sound Production in Two Sympatric Sibling Drosophila Species

Abstract. *Courting males of* Drosophila persimilis *produce a pulsed "vibration sound," with frequency of oscillation and pulse repetition rate markedly different from that of* D. pseudoobscura. *These differences probably contribute to reproductive isolation. Flight frequency is the same in both species. Brief "preliminary sounds" are common and the same in both species. Males with large parts of both wings removed produce nearly normal vibration sounds, and normal preliminary sounds.*

The range of *Drosophila persimilis* extends along most of the western coast of the United States and is almost entirely contained within the more southerly and easterly extending range of *D. pseudoobscura*. Compared with *D. pseudoobscura, D. persimilis* is generally found in cooler, more moist habitats and is relatively more active in the morning and less active in the evening. The two species have somewhat different food preferences. However, there is wide overlap in the natural occurrence of the two species; copulating intraspecific pairs of the two can be collected simultaneously from the same food source (*1*). Hybrids are extremely rare in natural populations (less than 1/1000). Though hybrids occur in the laboratory, partial sexual isolation persists. Thus there must be isolating factors effective at close range.

Differences in courtship acts are of special interest in this context. Vibration of one or both wings is a very common act in *Drosophila* courtship. In *D. persimilis* and *D. pseudoobscura*, as in *D. melanogaster* (*2*), this vibration is accompanied by the production of a train of sound pulses. Each pulse consists of two to seven almost sinusoidal oscillations, with amplitude decreasing from about the second oscillation (see Fig. 1). The frequency of oscillation and the pulse repetition rate differ markedly in the two species (see Table 1). No consistent differences between the two species were observed in the number of oscillations per pulse or the amplitude of oscillation.

The differences in the frequency of oscillation are not directly related to differences in the frequency of wing beat during flight, since these frequencies are the same in the two species and differ from the frequency of oscillation in "vibration sounds" produced by either species. The frequency of the wing beat during flight was determined stroboscopically for three 7-day-old males of each species. The range for *D. persimilis* was 185 to 215 cy/sec and for *D. pseudoobscura* was 185 to 219 cy/sec at 24° to 26°C. Reed *et al.* (*3*), using 3-day-old virgin females of several strains of each species, found that at 20°C, the wing beat of *D. persimilis* averaged 174 cy/sec and that of *D. pseudoobscura* averaged 182 cy/sec.

Shorey (*2*) found that the temperature in his closed recording cell increased by ¼° to ½°C per minute, and that the pulse repetition rate increased by 1.4 per second with each degree centigrade in the range of 25° to 30°C. My observations on the two species were made roughly in alternation on any given day of recording, so that both species were exposed to about the same range of recording conditions. Room temperature was kept within 24.5° to 26.5°C, and when a new pair of flies was put in the observation chamber, the air in the chamber mixed freely with room air. No trends were seen in pulse repetition rate in several records taken over a period of about 3 minutes. Thus, while cell temperature was not recorded it is virtually certain that the species differences in pulse repetition rate (and also frequency of oscillation) cannot be ascribed to variations in temperature.

The observed properties of courtship sounds are presumed to be characteristic of large portions of the natural populations of these species, since they were consistent for three strains of *D. persimilis* and two strains of *D. pseudoobscura* collected in widely different parts of California. Three of these strains had been kept in the laboratory for only two generations, one for four, and one for about 16 generations.

The second very commonly observed courtship sound (designated "preliminary sound") often directly preceded vibration, though sometimes it was not immediately followed by any courtship activity. It often occurred when the male was tapping the female prior to beginning vibration. I was unable to observe any movement which was correlated with the production of this sound. The sound may occur singly or in series, somewhat more rapidly than one sound per second. The tone of the sound is not pure and the oscilloscope shows a rather irregular waveform of about 4 cycles at 430 to 500 cy/sec.

The third courtship sound was comparatively rare. It occurred when males flicked both wings, usually as they approached a female from a distance. The sound produced by one flick is a burst of about seven oscillations of irregular waveform at about 155 cy/sec. Both males and females have been seen to flick both wings without producing detectable sound.

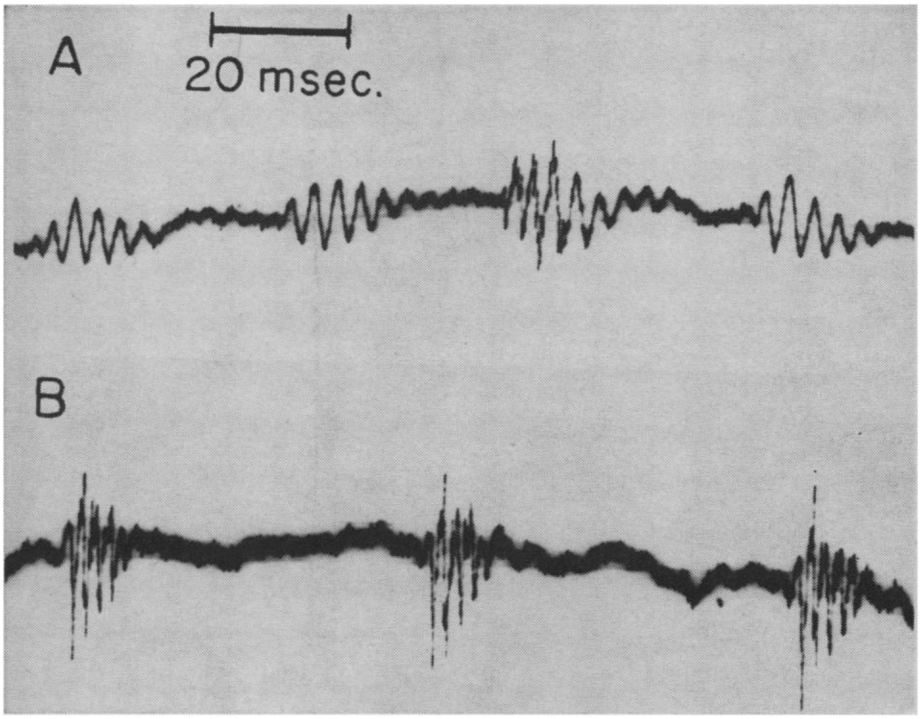

Fig. 1. Part of a train of pulses from a vibration sound from a *D. pseudoobscura* male (*A*), and a *D. persimilis* male (*B*). Filmed oscilloscopic records were made of the signal produced by a courting male walking on the diaphragm of a Wollensak crystal microphone, and amplified 500 times by a Tektronix preamplifier.

To explore the method(s) of sound production, I cut off varying lengths of the wings of a series of *D. persimilis* males. The wings of one male (No. 14) were initially 2.0 to 2.5 mm long; all but 0.1 to 0.15 mm of each wing was cut off. When placed with a female, this male began to court almost immediately, producing many preliminary sounds and a series of vibration sounds which were as loud, steady, and sustained as those produced by normal males, and of about the same pitch and temporal patterning. This indicates that motion of the air caused by the vibration of the wings is not a crucial factor in sound production, and further, that frequency determination is not critically dependent on the mechanical balance of the weight of the wings. Observations with other males supported both conclusions. However, in contrast to male No. 14, most other males produced preliminary sounds but not vibration sounds during an initial period of courtship, when both such sounds were always produced by normal males. In the males with various lengths of wings removed, this initial period was followed by a period of erratic vibration sounds which in most instances, developed gradually into nearly normal vibration sound production. The sound associated with wing flicking was not heard in the males with wings removed, suggesting that it may be produced by stridulation involving the wings. In normal males the vibratory sound was produced only in association with the vibration of the wing, so presumably the thoracic musculature participates in this type of sound production. The effects of wing removal indicate that the vibration sounds may be produced by the sound of the contraction of the thoracic muscles, transmitted to the substrate by the legs.

Table 1. Frequency of oscillation within pulses and length of interval between pulses in vibration sounds. All observations were made while the courting male walked on the diaphragm of a Wollensak crystal microphone. Oscilloscopic measurements were made from filmed records or by use of a storage oscilloscope. Aural frequency determinations are accurate to within ± ¼-tone. Intervals between pulses are measured from the beginning of one pulse to the beginning of the next.

Observations	Oscilloscopic measurements	
	D. persimilis ($N = 4$)	*D. pseudoobscura* ($N = 5$)
No. pulses observed	71	64
Intrapulse frequency (cy/sec)	479*	288†
Standard deviation	35	14
No. intervals observed	56	57
Interpulse interval (msec)	65	36
Standard deviation	23	16

* The pitch was aurally estimated for four additional males to be between A and B on the musical scale (440 to 495 cy/sec). † For six additional males, the pitch was aurally estimated to be between B and middle C (247 to 267 cy/sec).

The widespread occurrence of flicking and vibration in *Drosophila* courtship strongly suggests that these function in communication from the male to the female. Probably they serve to stimulate the female sexually or to identify the species of the courting male, or both. It is therefore of interest to consider the possible mechanisms of perception of vibration by the female. Vibration sounds might be transmitted through the substrate and perceived by chordotonal organs, two of which are found in each femur (*4, 5*). Autrum and Schneider (*5*) have shown that organs of this type in other insects are sensitive to vibrations of the substrate. Wing vibration may also, or alternatively, be a visual means of communication. The male usually vibrates while oriented with his head towards the middle of the female and his body rearward. The wing nearest the female's head is usually vibrated, so the vibrating wing is within the female's field of vision. The human eye can detect the species difference in pulsing frequencies. The work of Wolf and Zerrahn-Wolf (*6*) and Reichardt (*7*) indicates that the insect eye operates primarily as a frequency analyzer. Fermi (*8*) has shown that the flicker fusion frequency for *Musca domestica* is somewhat above about 450 cy/sec—that is, high enough to permit discrimination between 240 and 475 cy/sec.

Though we have obtained no direct evidence that these courtship sounds are of biological significance, the consistent differences between *D. persimilis* and *D. pseudoobscura* in frequency and pulse repetition rate of vibration strongly suggest that these differences play a role in maintaining reproductive isolation between these sympatric, sibling species.

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Cellular Mechanisms in Experimental Epileptic Seizures

Abstract. *Action potentials of single cells, recorded by means of extracellular microelectrodes from the cat's cerebral cortex after topical application of penicillin, undergo characteristic changes in the course of seizure episodes. In individual neurons, these changes in spike configuration are fully reversible and are repeatedly observed with each of such episodes. Two alternative interpretations offered for these findings appear to be critically dependent upon the relationship between the position of the microelectrode and the cell itself: there might be a transitory failure of the spike to invade part of the neuronal membrane or transitory neuronal swelling.*

A series of experiments was recently conducted for the purpose of analyzing the basic features of the epileptiform activity in the cerebral cortex (*1–3*). These experiments were performed on cats (*cerveau isolé* or light barbiturate anesthesia, or both) after discrete, topical cortical application of penicillin. The epileptogenic properties of this drug and other technical details were described previously (*1, 4, 5*). Both intra- and extracellular recording were obtained from the population of cortical elements in the affected area.

Here we report an interesting aspect of findings which was not included in the main account of our study (*1, 2*). Several interpretations are possible, and two of these are discussed in relation to the mechanisms for the development of the rhythmical self-sustained discharge which characterizes the epileptic seizure.

Soon after cortical application of penicillin, isolated paroxysmal events (Fig. 1, *A, E*) began to appear, consisting of typical electroencephalographic and single cell behavior patterns (*1, 5*). These, which can be considered the experimental equivalent of inter-ictal (subclinical) events of human epilepsy, kept recurring spontaneously for many hours or could be "triggered" by various types of electrical stimuli (direct cortical or callosal stimulation, or stimulation of the specific projection pathways) at critical frequencies (*3, 6*). On occasions, the gross and unitary activity underwent characteristic changes (Fig. 1, *B–D*) and assumed the form of long lasting, ictal episodes (*2*).

The behavior of 56 cortical units could be studied repeatedly in the course of several of such ictal episodes by means of extracellular electrodes. This study was carried out either by photographing on moving film the cathode ray oscilloscope tracing of both gross surface cortical and unitary changes (Fig. 1), or with photographs of single sweeps of the oscilloscope. By this second method, the same changes could be analyzed in detail as shown by the samples selected at given intervals before, during, and after the course of the ictal episode, the entire episode being monitored, in parallel from the same input, in inkwriting tracings (Fig. 2). When this method was used, it was convenient to trigger the sweeps (and the epileptiform events) with brief electrical pulses applied locally or to the contralateral homologous region of the cortical surface. The phenomena to be described, however, did not seem to be altered by, or depend upon such stimulation and were repeatedly observed also in its absence (for example, see Fig. 1).

In 29 of these 56 cells it was found that, in the bursts of spikes of modulated (Fig. 1*A*) or of progressively decreasing amplitude (Fig. 2*A*), which characterize the inter-ictal behavior of the "epileptic" neuron, the action potentials consisted of a diphasic (positive-negative) deflection. In the course of the ictal episode, however, these same spikes would undergo a relatively constant modification, losing their diphasic character, due to a partial (Fig. 1, *C, D*) or total (Fig. 2, *B, C*) disappearance of the second, negative phase. In addition, during the episode a slow positive potential would appear in coincidence with the spike firing (Fig. 1, *C, D*); if already present this potential would become more prominent (Fig. 2) or, if a similar, negative potential was appreciable before the ictal episode, its polarity would reverse. These and other changes (shortening of latency when the spikes were activated by electrical pulses; increase in the duration of individual spikes), which are not taken into consideration here, were reproducible in the course of different ictal episodes for any given one of the 29

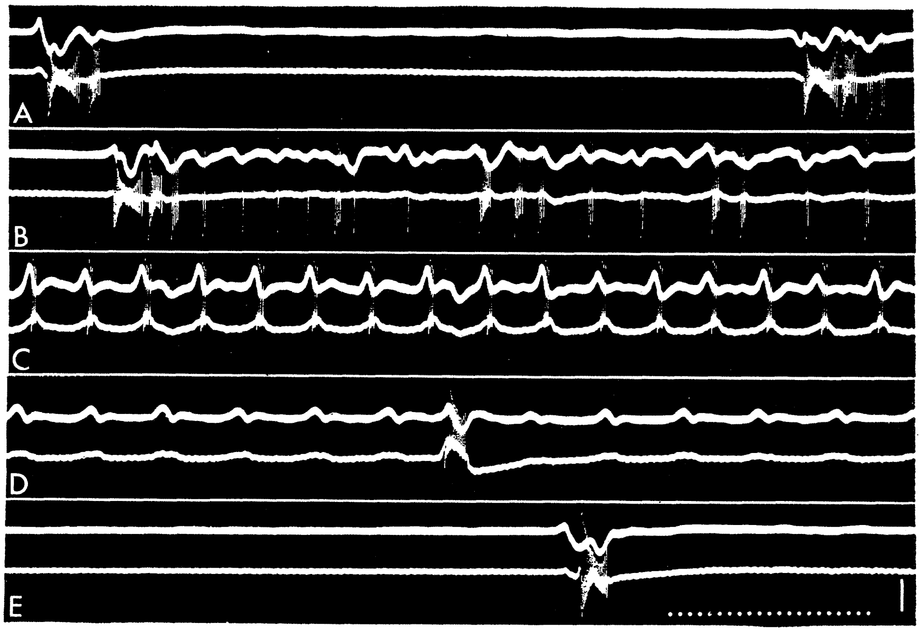

Fig. 1. Records from gyrus sigmoideus posterior close to site of penicillin application. In each pair of tracings the upper one is from gross surface electrodes (a-c amplification), the lower one from an extracellular micropipette (d-c amplification). The five sections are separated by the following intervals in seconds: *A–B* (0.5); *B–C* (11); *C–D* (8); *D–E* (23). *A,E*: "inter-ictal" manifestations; *B–D*: "ictal episode." Positivity is indicated by upward deflection. Calibrations: 1.5 mv (upper tracing) and 3 mv (lower tracing); 25 msec.