experiments but it is clear from the figure that the animals had accumulated copper and that the concentration of this metal virtually doubled during the 10 weeks of these experiments. The presence of molybdate/sulphate supplements did not influence the ability of snails to take up copper even though sulphate-reducing bacteria were present in their crops. There is, therefore, no evidence of the complex copper-molybdenum-sulphate interactions that occur in ruminants and which have been interpreted as copper deficiency diseases resulting from the bacterial synthesis of thiomolybdates or the precipitation of copper sulphides[7]. In fact the copper content of the snails fed supplemented diets appeared to be greater than those of controls (fig.). One explanation for this seems to be that all the snails on these supplemented diets were found, at the end of the experiment, to contain sulphate reducing bacteria whereas these bacteria occurred in only 50% of the controls. This suggests that the presence of sulphate in the diet encourages the growth of these bacteria in the animals crop. In addition if the copper analyses of the control snails are considered in relation to the presence or absence of bacteria (table 2) it becomes clear that not only is there a significant difference between these groups but the snails with the bacteria have a higher copper content that is equivalent to those fed molybdate/sulphate diets. This indicates that far from being inhibitory the sulphate reducing bacteria are actually beneficial and increase the availability of copper to the snail presumably by the effects of their own metal chelating secretions. This raises a number of interesting questions. Firstly, although the digestive tracts of many molluscs are known to contain bacteria it is only the sulphate reducers that have been considered in these experiments. Does this imply either that these bacteria are good indicators of total bacterial populations or, as seems more likely, that sulphate reducers are particularly good at making copper available to their hosts? Second, is the effect specific for copper or are other metals such as zinc also made more available to the snail? Finally, is this part of a general phenomenon i.e. that those invertebrates that lack an acidic stomach may facilitate their uptake of trace metals by absorbing bacterial metal-chelate complexes in a way analogous to plant root systems. Certainly it appears that ecologists who use invertebrates as environmental monitoring systems should recognize that particular metals may be made more available to these animals by bacterial faunas which may themselves vary with the occurrence of certain pollutants.

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## Nature of the sound produced by courtship-inhibiting behavior of the male *Drosophila mercatorum*[1]

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*Summary.* The male of *Drosophila mercatorum* was found to produce a courtship-inhibiting sound when he was courted by another male. The nature of the sound was significantly different from the courtship sounds emitted by a courting male.

*Key words. Drosophila mercatorum;* courtship sound; courtship-inhibiting sound; courtship-inhibiting behavior; male-male interaction.

The role of courtship sounds (songs) released by wing vibration of a courting male is known to act positively as a species recognition signal and/or as a sexually stimulating signal that lowers the threshold level of female receptivity[2–5]. It has also been shown in some drosophilid species that the female produces sounds in response to male courtship[2,6,7]. Some of these are thought to act as a signal of rejection by unreceptive females, resulting in inhibition of further male courtship. The author reports here that when one male courts another, the latter produces a unique sound that results in inhibition of the courtship of the first male. Such a sound, produced during male-male interaction, has not been described previously in *Drosophila*.

*Materials and methods.* The wild type laboratory strain of *Drosophila mercatorum* used was R (Rochester, New York, collected in 1957). The male of *D. mercatorum* displays two kinds of courtship behavior accompanying wing vibration, which consequently produces two kinds of courtship sound. The first (A sound) is produced by wing vibration and wing flicking by the male when positioned behind or by the side of the female. After producing the A sound, the male approaches much more closely behind the female, extends one wing at about 40°, and vibrates both wings. The sound thus produced is referred to as the B sound, after which the male finally attempts to mount. The results obtained so far suggest that the A sound may be essential for male recognition by the female and that the B sound is more closely associated with copulation itself[3].

The flies used were raised and aged on the standard *Drosophila* medium seeded with live yeast. They were kept at 25 ± 1°C on a 12-h light-dark cycle. Mating tests and recording of the sounds were carried out during the beginning of the dark period, at 6 days of age. This species is known to be sexually most active in the evening, and at this age[8].

Observations of mating behavior were made at 10 × magnification. Procedures both for recording sounds and for obtaining oscillograms have been described elsewhere[3]. Interpulse intervals of the sounds were determined from the oscillograms. Sonograms were produced using a Kay Electronic Co. 7030A sound spectrograph with the reproducing circuit of the sonograph set at 20–2000 Hz. The relative quantification of sound levels was made by a sectioner analysis which permits the individual intensity of each frequency component to be displayed at any preselected point in time; this permits one to estimate both a funda-

Nature of the three kinds of sounds produced by males of *D. mercatorum*. The mean values are given with standard error. IPI, interpulse interval; PPB, the number of pulses per burst

| Sound | Oscillogram analysis | | | | Sonogram analysis | | |
|---|---|---|---|---|---|---|---|
| | No. of males tested | No. of IPI measured | IPI (ms) | PPB | No. of sonograms examined | Fundamental frequency (Hz) | Dominant frequency (Hz) |
| Courtship sound | | | | | | | |
| A sound | 6 | 43 | 9.3 ± 0.1 | 8.2 ± 0.6 | 5 | 110.8 ± 0.8 | 550 |
| B sound | 10 | 50 | 57.4 ± 0.6* | 20.2 ± 2.3* | 5 | — | 521.4 ± 13.6 |
| Male courtship-inhibiting sound | 6 | 37 | 40.4 ± 0.7 | 7.2 ± 1.4 | 5 | — | 563.4 ± 5.8 |

* Data from Ikeda et al.[3].

mental frequency and a dominant frequency. The average values of the nature of the sound employed in the present study were obtained as the mean record from 5–6 individuals for each sound.

*Results and discussion.* In *D. mercatorum*, a male was frequently observed to be being courted by another male that was producing the A sound. The male-male courtship happened rapidly, not exceeding 1 sec. The male being courted by the other male frequently tapped the other with his fore- and middle legs and emitted sound pulses (fig. 1). These were accompanied by flicking and vibration of both wings which were slightly extended (about 10°). The courting male then immediately went away from the other male and did not follow him. The pulse sound produced by the male being courted appears to be effective in inhibiting subsequent courtships. Accordingly, this sound can be referred to as a male courtship-inhibiting sound (MCIS).

The courtship-inhibiting wing movements and the nature of the sound (table) are clearly different from those of the courtship A and the B sounds; the B sound has never been recorded during male-male courtships. There are apparent differences in the wave pattern both of the oscillograms (fig. 1) and the sonograms (fig. 2) among the three sounds produced by the male during courtships. Five sonograms were quantitatively examined for each of the three sounds. The A courtship sound is clearly harmonic with a fundamental frequency of 110 Hz on the average and give a dominant frequency of about 550 Hz. The courtship B sound and MCIS seems to consist of closely spaced frequencies; however, no fundamental frequency can be determined. The average dominant frequencies are 521 Hz for the B sound and 563 Hz for MCIS, significantly differing from each other at the 5% level (t = 2.83).

According to the oscillograms, two quantitative measures are available for comparative study. One of them is the average interpulse interval, IPI, i.e., 'the average length (in ms) from the beginning of one pulse to the beginning of the next one'[2]. A second measure is the average number of pulses per burst (a pulse train), PPB. An approximate length of a pulse train can be estimated by an IPI (ms) × PPB calculation. The measures of the A sound and MCIS were obtained on the basis of observations of six male-male courtship events; the values of the B sound were those given by Ikeda et al.[3]. The average IPI of MCIS was 40.4 ms, significantly differing both from the value of the A sound (9.3 ms) recorded for the same males ($p < 0.001$, $t = 50.70$) and from the value of the B sound (57.4 ms) ($p < 0.001$, $t = 18.55$) which was obtained for the first five pulse intervals of the B sound[3].

The PPBs of MCIS varied from 3 to 11 in the present records, giving an average of 7.2 pulses for each event in which a male was repelled. The average PPBs were 8.2 for the A sound and 20.2 for the B sound[3]. Thus the length of one pulse train was approximately 290 ms, 78 ms and 1102 ms for MCIS, A and B sounds respectively.

What kinds of different signals are transmitted during inhibition of male-male courtship? It is well known in *D. melanogaster* that the adult male produces a volatile pheromone which inhibits courtship between males[9]; the mated female also emits a courtship-inhibiting pheromone[10]. The chemical nature of these evidently differs. Other possible chemicals may consist of courtship-inhibiting contact pheromones which can be directly transmitted through tapping betweenm individuals. However, there is no direct evidence for this possibility.

In the case of the male-male courthsip in *D. mercatorum*, two other possible cues may be pointed out. The special wing movements may act both as visual and auditory signals in courtship-inhibiting cues. The male-male courtships were very short even in the dark (these latter observations were made in red light). Thus it is possible that the visual cue may not be necessary to inhibit courtship. The sound produced by the male being courted regularly had an amplitude as high as that of the courtship sound. The courtship was immediately terminated after the emission, or reception, of MCIS. Considering these facts, it is concluded that the auditory cue released by wing flicking-vibration of the male may be important in inhibiting the male-male

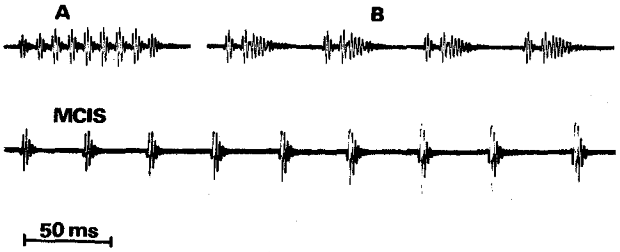

Figure 1. Oscillograms of the three kinds of sounds produced by the male in *D. mercatorum*. A, the courtship A sound; B, the courtship B sound; MCIS, the male courtship-inhibiting sound.

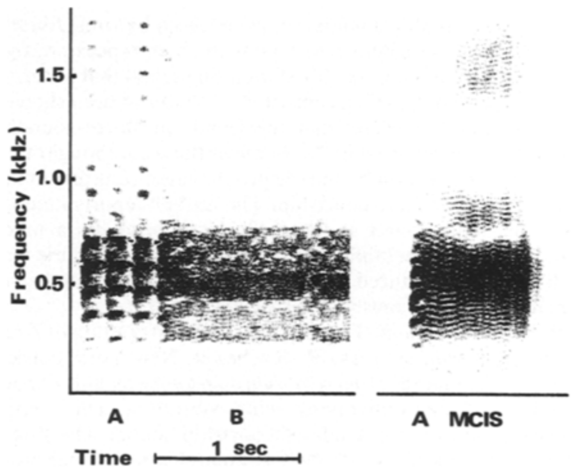

Figure 2. Sonograms of the three kinds of sounds produced by the male in *D. mercatorum*. A, the courtship A sound; B, the courtship B sound; MCIS, the male courtship-inhibiting sound.

courtship in *D. mercatorum*, although the existence of contact pheromones is highly probable since the courted male frequently taps the other male with his fore- and middle legs during their encounter.

The male may be first sexually stimulated by essential signals such as sex pheromone emitted by the female[11–13]. However, observations both in the laboratory and in the field suggest that the male, having once been excited, seems to try rather randomly to contact any individual that is near at hand. He is then capable of determining his proper sexual partner, mainly by using the sex pheromones as a clue. On the other hand, the male being courted by another male should be freed as soon as possible from annoying encounters and play his normal male role. This situation is also true for the male that improperly courts other males. Thus the courtship-inhibiting cues such as the chemical and the auditory ones are very useful under natural conditions in avoiding wasted time at feeding sites where many individuals assembled and mate.

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## The effect of head heating on the flight activity of the cockroach

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*Summary.* Internal head heating restricted the flight activity in the cockroach *Periplaneta americana*. Heating the metathoracic ganglion did not have any pronounced effect on the flight. It is concluded that in the cockroach the head nervous centers are very heat-susceptible and might be the coordinating center for temperature responses.

*Key words. Periplaneta americana;* head temperature; flight activity.

Studies performed on homeotherms have given evidence that in the exercising animal the brain is the part of the body most protected against overheating[1–5]. From the point of view of comparative physiology it is interesting that also in the honeybee *Apis mellifera* the ability to fly at air temperatures up to 46 °C is accompanied by a very effective head cooling mechanism[6,7]. However, most insects must limit their activity to avoid overheating in a hot environment[8]. This is particularly true of species which do not exhibit any physiological thermoregulation, for example the American cockroach *Periplaneta americana*[9]. The aim of the experiments presented was to verify whether increased head temperature was a limiting factor for flight in *P. americana*.

*Materials and methods.* The experiments were performed on adult males of the American cockroach *Periplaneta americana* weighing 997 ± 12.53 mg. The insects were kept under natural photoperiod at 28–30 °C and 55–65% relative humidity. They were fed on a diet of apples, porridge and water ad libitum.

Thermodes made from loops of enamel-insulated constantan wire, 0.05 mm in diameter, were used for internal body heating. The loops were extended by copper leads to a DC power supply unit. The body was heated as the current flowed through the high-resistance constantan wire. The temperature of each thermode was measured by a thermocouple fixed to it with polyethylene glue. The copper-constantan thermocouples were made from wires 0.05 mm in diameter. They were calibrated with the use of a Beckman thermometer and a digital nanovoltometer (type 180, Keithly Instr. Inc., USA). During the experiments the temperature was recorded on a Speedex Recorder SP-G7P (Riken-Denshi Co. Ltd., Tokyo, Japan). Its sensitivity was 0.089 °C per 1 mm pen deflection. The measuring system was calibrated by means of a reference voltage from a thermocouple placed in a water thermostat, which stabilized the temperature at ± 0.05 °C.

The thermode/thermocouple pairs were implanted into the animal from its dorsal side, the first one 3 mm deep into the head and the other into the metathorax near the ganglion. They were fixed with Taki Wax.

After the implantation only cockroaches which flew well were used for experiments. Ten were selected out of 15. The selected insect was placed in chamber (70 × 50 × 50 cm), which was heated with a warm air stream. The temperature in the chamber (recorded by the thermocouple) was stabilized at 28.4 ± 0.06 °C. In the chamber the animal stayed at rest for about 30 min. After that time it was forced to fly, as the body lifted on the thermocouple leads had no contact with the ground. When the wing motion stopped, the insect was set down for a second and then lifted again, which acted as a stimulus to continue the flight. Every stimulus was automatically recorded on a tape recorder. The stimulation was stopped when no response was observed. The maximal time for stimulation was 10 min. The flight was not interrupted if it was continued after the 10th min; only after the cessation of flight was the animal set down.

The body heating started immediately after the animal had been placed in the chamber and followed at a rate of 0.6 °C/min. After the desired level had been reached, the temperature was stabilized.

The following parameters were used to describe flight activity of the cockroach: 1) frequency of stimulation, 2) period of positive responses to stimulation – later called 'duration of flight', 3) maximal increase of thorax temperature (MTT).

Each experimental series was performed on 10 insects. For each calculated parameter the mean values and standard errors are shown.

The experiments were conducted during the day time.

*Results. Control.* When neither the head nor the thorax was heated the cockroaches had to be stimulated to flight 4.8 ± 1.4