are not as discretely separate as in well-studied *D. melanogaster* larvae where the salivary glands secrete glue proteins prior to the time of pupariation, a time gauged by the wandering stage[26]. Metamorphosis is delayed till spring. Hence other substances for cryoprotectants may have to be manufactured from stored products, as has been found for *E. solidaginis* larvae[27]. For instance, alanine is high in the dead *C. amoena* larvae, in live larvae it is depressed below control (22 °C) values while proline accounts for over 50% of the free amino acids in the hemolymph.

However, mortality remains high. One or fewer than one larva per substrate ecloses[9]. Only 27 adults completed development from the 33 walnuts held to May 1985. Reminiscent of the genetic load arguments of the 1960s[28], this suggests that seasonal changes might also involve genetic shifts between summer and winter.

Phosphoglucomutase regulates the flow of glycogen in and out of the fat body. The decline in the frequency of $PGM^F$ in late fall and its increase again after spring to 70% in summer is in agreement with findings for other drosophilid species that selection operates at this locus but that geographical clines do not exist[29]. Other studies have shown a relation between the biochemical properties of enzymes and the behavior of genes in natural and laboratory populations[30].

The fat body is reconstituted in adult *Drosophila*. From the difference between *D. melanogaster* and *C. amoena* larvae at room temperature, it is clear that proline regulation has been affected in drosophilid evolution. Only trace quantities of sorbitol have been found in adult *Drosophila*[7]. Whether or not proline functions in cold hardiness in the genus *Drosophila* remains to be determined.

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## Ultrasound: its role in the courtship of the arctiid moth, *Cycnia tenera*

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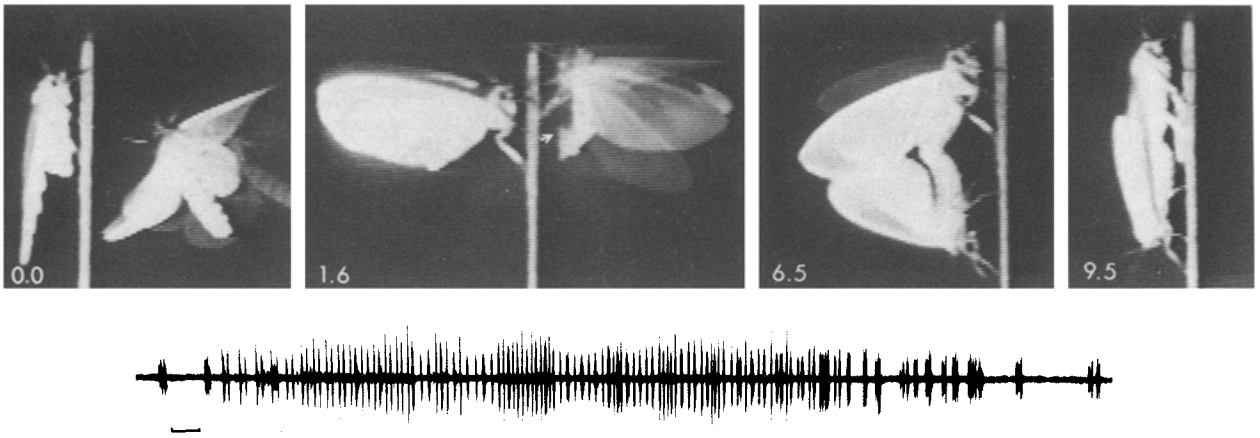
*Summary.* Male *Cycnia tenera* (Lepidoptera: Arctiidae) were shown to produce ultrasonic clicks during courtship. The ultrasound enhances male courtship success, but only in the absence of male-produced pheromonal cues.
*Key words.* Ultrasound; pheromone; coremata; Arctiidae.

Moths were first shown to be sensitive to ultrasound in the seminal experiments of the late Kenneth Roeder. He and his collaborators showed that moths detect the ultrasonic echolocation signals produced by bats and evade bats by responding both directionally and non-directionally to these signals[1-4]. Later, arctiid moths were found to produce ultrasound using tymbal organs located on the thorax[5,6]. Whether they use these sounds to startle bats, to jam their sophisticated echolocation systems, or to 'advertize' their distastefulness (i.e. produce aposematic sound) is still under debate[7-10]. I here present evidence that ultrasound in arctiids plays yet another role: in the dogbane tiger moth, *Cycnia tenera,* it serves in the communication between the sexes.

Dogbane tiger moths are known to produce trains of ultrasonic clicks in response to bat cries and when handled[6,11,12]. Clicks are produced by the buckling and relaxation of striated, air-filled blisters of cuticle (thoracic metepisterna) called tymbal organs[5,6,13]. Each flexion/relaxation cycle produces a doublet of clicks referred to as modulation half-cycles[14]. Repeated doublets form a train. The clicks are faintly audible to humans, but most of the sound energy is ultrasonic with a peak near 50 KHz[6,9,11]. Similar trains of clicks are produced by male *C. tenera* during courtship suggesting they function as intersexual signals (fig.). The following is an investigation of their role in courtship, a role first suggested for the moth-produced clicks in 1864 by Laboulbene[15].




*Top*: Typical courtship sequence videotaped using synchronized deep red strobe (camera, Panasonic WV-1850; videorecorder, Panasonic NV 8950; strobe, GenRad electronic strobe GR 1531-AB). Timing of interlaced video fields shown relative to initial approach of male (first photo) in seconds. Pheromone disseminating structures (coremata) are visible (arrow). *Bottom:* Recording of ultrasonic clicks produced during typical courtship sequence (Hewlett Packard 3960 instrumentation recorder; sound slowed to 1/1024 × normal speed). Ultrasonic microphone (± 1.5 db from 1 to 100 Khz). Instrumentation recorder (± 3.0 db from 0.1 to 60 Khz). Time marker = 100 ms.

*C. tenera* were raised outdoors through their final larval instar on a stand of Indian hemp, *Apocynum cannibinum*. Larvae were transferred into a laboratory incubator (27 °C; 16L:8D photoregime) for pupation and eclosion. The courtship of *C. tenera* (2–7 days old) was videotaped (fig.) in a laboratory windtunnel (60 cm × 60 cm × 150 cm; windspeed 25 cm/s). Stationary females released their volatile sex pheromone and stimulated males to fly upwind, locate the female, and mate directly in front of the video camera. The ultrasonic clicks produced during courtship were recorded using an ultrasonic microphone and instrumentation recorder. The ultrasound was also fed through an envelope detector resulting in the production of audible clicks which were recorded on the audio track of the videotape. The mating capabilities of six groups of males were compared: normal males, males deprived of the use of their courtship pheromone, aphonic males, males without pheromone and sound, and control males. Males were deprived of the use of their courtship pheromones by using a cyanoacrylate ester-based glue to fasten the eighth and ninth abdominal sternites together. This effectively prevents the eversion of their air-inflatable scent disseminating structures called coremata. Such structures have been shown to release courtship pheromones in related species[16–18].

Males were rendered aphonic by encasing the tymbal organs in a quick-drying water soluble glue. Such males produced no detectable ultrasound under conditions when they normally do (when they are handled or during courtship). Control males had comparable amounts of both glues applied to the eighth abdominal sternite and to a noncritical portion of the tymbal organs, respectively.

In the courtship of normal and control males ultrasound production began at or in many cases prior to initial contact between males and females. Ultrasound production continued usually as one continuous train of doublets until the genitalia were engaged (mean duration of sound production = 5.8 ± 2.0 s for 8 normal males and 6 control males for which complete data were available). Courtships involving aphonic males were silent. This and a click-by-click timing analysis that revealed a train of regularly spaced doublets with few interjections indicated that almost all sound in courtship can be attributed to the male. Males without sound and males without pheromone suffered no apparent mating disadvantage. However, the mating success of males without both pheromone and sound was significantly depressed relative to all other groups (table). Females evaded males without both cues and in three cases actually flew away from their perch. These results indicate that either the production of ultrasound or the eversion of the pheromone disseminating structures is sufficient to ensure copulation.

The precopulatory sexual interactions of moths have been interpreted in several contexts, i.e. in the context of reproductive isolation, female mate choice, and male-male competition for mates[19]. Ultrasound production by male *Cycnia tenera* may function in one or more of these ways. Whatever the ultimate function(s) of the moth-produced ultrasound it is clear that acoustic signals play a greater role in moth courtship than previously suspected. Ultrasound has been known to be an important parameter in the courtship of the greater and lesser wax moths (*Galleria mellonella* L. and *Achroia grisella;* Pyralidae)[20–22], but in these species it was thought be a communication signal unique to moths that live in natural cavities (honeybee nests). In light of the results presented here, moth courtship must be reevaluated with greater emphasis on the interaction of multiple modalities, especially the chemical and acoustic.

Current studies by others[23] on two arctiids, *Phragmatobia fulginosa* and *Pyrrharctia isabella*, in which the female emits ultrasound in response to pheromone-stimulation by the male have failed to reveal a clearcut role for the sounds. Deafened males oriented well to females in field experiments and silent females showed no mating deficit.

Influence of removal of sound-producing and pheromone-releasing ability on courtship success

| Treatment | % Successful (#) | % Unsuccessful (#) |
|---|---|---|
| Normal males | 100 (25) | 0 (0) |
| Males without sound | 90 (9) | 10 (1) |
| Males without pheromone | 100 (15) | 0 (0) |
| Males without sound and pheromone | 57 (7) | 43 (5)* |
| Control males (sham-operated) | 100 (11) | 0 (0) |

*Significantly different ($p < 0.001$) from other groups combined (Fisher Exact Probability Test).

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## Heat production by balling in the Japanese honeybee, *Apis cerana japonica* as a defensive behavior against the hornet, *Vespa simillima xanthoptera* (Hymenoptera: Vespidae)

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*Summary.* As an effective counterattack strategy against predacious hornets, especially *Vespa simillima xanthoptera,* workers of *Apis cerana japonica* showed a distinct balling reaction, usually involving 180–300 bees. This produced heat for as long as 20 min, giving rise to temperatures inside the ball higher than 46 °C, which is lethal to the hornet but not to the bees.
*Key words.* Heat production by balling; defensive behavior; *Apis cerana japonica;* the Japanese honeybee.

The hornet, *Vespa simillima xanthoptera* Cameron (Hymenoptera: Vespidae) is a major natural enemy of the Japanese honeybee, *Apis cerana japonica* Radoszkowski (Hymenoptera: Apidae). From July to October, the worker hornets appear in the vicinity of the hive (or nest in the natural habitat) entrance of the honeybee, and hunt the honeybees to feed to their larvae. However, the predators are sometimes captured by the honeybees and killed by engulfing or balling (fig. 1).

Thirty-six ballings were observed at hive entrances from August to September in 1984, 1985 and 1986 at Machida-shi, Tokyo, Japan. In 20 of them, the temperature inside the ball was monitored by using a thermometer (Model R116, Takara Kogyo Co. Ltd.) fitted with 3 micro-thermistors (O.D. 1 mm, Model TZL-64). The micro-thermistors were placed: 1) inside the ball; 2) in the center of the honeybee colony; and 3) beside the hive (recording the ambient temperature). An example of the results is shown in figure 2. As soon as the balling started, the temperature inside the ball increased rapidly and reached more than 46 °C within the first 4 min. After the temperature had been maintained for about 20 min, the temperature slowly fell until it was the same as that of the central part of colony (ca 34 °C). After that, the temperature of the ball rapidly dropped to the ambient level again. The average maximum internal ball temperature was 46.1 °C (range 45.2 °C to 47.0 °C) in the 20 balls observed.

The behavioral sequence was also observed. Within 15 s after the counterattack by the first guard bee, many others simultaneously rushed onto the captured hornet. As a result, the hornet was engulfed by some 250 worker honeybees (ca 180–300). The number of bees balling the hornet was usually constant for more than 30 min, and then gradually decreased. When the number of balling bees was reduced to about 10, the dead hornet was visible together with some bitten honeybees. Guard bees did not pay strong attention to the dead hornet, and some of them removed it by carrying it in their mandibles. The average duration of the whole sequence was 60.7 min (range 40.1 min to 108.5 min). We observed that all the hornets were killed without exception. No honeybee sting was found in the hornet corpses and the hornet wings were sometimes curled by the produced heat (fig. 1D). The honeybees in the ball did not sting even if it was put on the palm of the hand (fig. 3).

Although a similar balling reaction has also been observed in introduced European honeybees (*A. mellifera* L.), the workers readily use their stings against the hornet during balling. Because of their sting autotomy, 2 or 3 stings usually remain in the intersegmental membrane of the dead hornet. The average maximum temperature inside the ball was 42.8 °C, which is significantly lower than that of the native Japanese honeybee. This appears to be related to the less frequent use of the sting by the Japanese honeybee in defensive behavior than in the European honeybee[1,2].

To verify the above-mentioned observations, upper lethal temperatures for both the Japanese honeybee and the hornet were compared in the laboratory. Fifty honeybees were collected from the actual ball and the same number of hornets were collected from those visiting the apiary. Individuals were put in 100-ml flasks. The internal flask temperature was raised at a rate of 2.7 °C/min by immersion in a constant-temperature water bath. The results showed that the upper lethal temperature for the Japanese honeybee was 48–50 °C, but that for the hornet was 45–47 °C. This result indicates that the Japanese honeybee can kill the hornet at the temperature produced by balling, without stinging.

Evidence of defense by heat production in poikilothermal animals has not been found previously and it is very interesting from the ecological and evolutionary points of view in insect societies.