

## Antennal twisting as courtship behavior in a local blister beetle, *Epicauta hirticornis* (Coleoptera: Meloidae)<sup>1</sup>

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Yien-Shing Chow, Rong Kou, Ru-Shiow Tsai, Hsiao-Yung Ho (1992) Spiral complex of insect antennae. *Bull. Inst. Zool., Academia Sinica* 32(1): 82-86. The blister beetle, *Epicauta hirticornis* Haag-Rutenberg, shows unique pre-copulating behavior. When males dorsally mount females, their antennae continuously wave to female antennae; often the male's right or left antenna grasps on the corresponding female antennae, thereby forming a double helix. The frequencies of establishing right- or left-handed antenna spiral complexes are equal. If the female's left antenna cannot be grasped, the male will form a spiral with female's right antenna, and vice versa. When both male and female antennae are entwined, they vibrate at a frequency of 7-12 c/s. This vibration is similar to the precopulatory sexual display of wing fluttering by male moths. The blister beetle's connection with cantharidin is also discussed.

**Key words:** Blister beetle (*Epicauta hirticornis*), Copulatory behavior, Double helix structure, Cantharidin.

From atoms, molecules such as L-amino acids and B DNA (Hegstrom and Kondepudi, 1990) to living organisms, nature is asymmetric with respect to chirality, or left- and right-handedness. Chiral symmetry and asymmetry can be demonstrated experimentally in a laboratory. When sodium chlorate (NaClO<sub>3</sub>) is crystallized from an unstirred aqueous solution, equal numbers of levo and dextro NaClO<sub>3</sub> salt are found. However, when the solution is stirred, almost all of the NaClO<sub>3</sub> salt found in a particular sample had the same chirality either levo or dextro (Kondepudi *et al.*, 1990). We used the twisted

antennae of courting male and female blister beetles, *Epicauta hirticornis* Haag-Rutenberg, to demonstrate that this phenomenon not only occurs on the molecular level but also in the behavior of living organisms. In June 1989, at a local mountain area near our Institute, numerous blister beetles were found aggregating on the leaves of flower glory-bowewer tree, *Clerodendrum cyrtophyllum* Turcz., and a novel sexual behavior was observed. During courtship, a male beetle mounts a female and his antenna winds about hers to form a double helix from behind. The sexual behavior of Meloidae was first reported by Selander (1964), and has subse-

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quently been studied in detail by Pinto (1975), Pinto and Mayer (1986), Brown (1990), and Yang (1991). The typical sexual behavior of many North American species of the blister beetle includes both male antennal fanning and the male's use of its antennae to repeatedly pull the female's antennae in and out of two corresponding grooves on the male's head (see Pinto, 1975; Yang, 1991). The entwining of male and female antennae to form double helix structures has not been previously reported. We report here a new type of sexual display, namely the formation of double helix of the male and female beetle's antennae in the oriental species *Epicauta hirticornis* which is an occasional pest of legume crop in Taiwan.

## MATERIALS AND METHODS

In May, 1990 a large population of the blister beetle *Epicauta hirticornis* was found feeding on both flowering glorybower trees and the leaves of the pteridophytes *Histiopteris incisa* (Thunb) and *Dennstaedtia scandens* Moore. Fifty adults were collected; individuals of both sexes were maintained in plastic cages (30 × 30 × 30 cm<sup>3</sup>) along with collected amounts of their host plants. Sexual behavior was recorded and analyzed with the aid of a video camera (National S-VHS movie, AG-410, Japan).

Then, in June, 1991 25 females and 30 males were collected again from the same location. Beetles were disturbed with forceps, which caused them to secrete a yellowish fluid from the joints of their forelegs; samples of this fluid were collected with capillary tubes. The chemical composition of the fluid was analyzed using standard gas chromatography and a mass spectrometer (Varian 3400 and INCOS 50, Finnigan MAT) with DB-1701 column. Benzophenone was used as an internal standard.

## RESULTS AND DISCUSSION

During courtship the male beetle mounts the female and winds his antenna around her corresponding antenna to form a double helix structure with 3-5 twists (Figs. 1a and 1b). We observed that males usually fan their antennae towards female antennae at a frequency of  $7.1 \pm 1.2$  times/sec. If the male achieves contact with his antenna and hers, he will use his right or left antenna to grasp her corresponding antenna, and the pair of antennae form a double helix (Fig. 1b). The observed frequencies of right-handed and left-handed antennal pairs were not significantly different (Table 1). We found that females could avoid mating by pressing their antennae against the surfaces of host plants or by hiding their antennae. If one of the female antennae is not available, the observed ratio of left- to right-handed configurations remain close to that for the available antenna.

These observations suggest that the formation of the antennal double helix occurs randomly with respect to 'handedness' which is similar to the formation of crystal in an undisturbed sodium chlorate solution (Kondepudi *et al.*, 199). Once male and female antennae are held in a tight helical configuration, the paired antennae vibrate at a frequency of 7-12 c/s for about 30-120 sec. However, in most cases the burst of continuous antennal vibration lasts for 30-60 sec.-similar to sexual display of wing fluttering in the diamondback moth (Chow *et al.*, 1974) and American cockroach (Chow and Wang, 1981). In the blister beetle, this courtship display concludes with copulation (Fig. 1c). Four periods of copulation 65, 78, 70 and 92 min., with  $\bar{x} = 76.25 \pm 11.78$  min. were recorded in our laboratory.

In the entire mating process, we have not observed any sign of raising or vibrating of the wings as those observed in many other insects. Developmentally, antennae and wing appendages are homologous units and wing



Fig. 1. Courtship behavior of the blister beetle *Epicauta hirticornis*.

- a. A male mounts a female feeding on the leaves of *Clerodendrum cyrtophyllum*. Their antennae are twisted to the right.
- b. Both male antennae are entwined with the female's antennae; right-handed and left-hand structures are seen pointed backwards.
- c. Normal copulatory position. The female is on the left.

Table 1. Observed frequencies of left-handed (LH) and right-handed (RH) antenna helixes in seven pairs of blister beetles. The simultaneous formation of both right and left-handed (LH + RH) helixes is also shown. LH + RH helixes occur at much lower frequencies. For each observation, counting time lasted 30 minutes; the data represents an average of six repeated occurrences.

Pairs of Insects observed	LH (%)	RH (%)	LH + RH (%)
1	11 (35.5)	17 (54.8)	3 (9.7)
2	23 (69.7)	9 (27.3)	1 (3.0)
3	19 (48.7)	17 (43.6)	3 (7.7)
4	27 (56.3)	17 (35.4)	4 (8.3)
5	10 (40.0)	15 (60.0)	0 (0.0)
6	19 (48.7)	14 (35.9)	6 (15.4)
7	9 (28.1)	18 (56.3)	5 (15.6)
$\bar{x} \pm SE$ (%)	16.9 $\pm$ 7.0 (46.72 $\pm$ 13.9)	15.3 $\pm$ 3.1 (44.8 $\pm$ 12.5)	3.1 $\pm$ 2.1 (8.5 $\pm$ 5.8)

disc could be transdetermined into antenna as it has been demonstrated in *Drosophila melanogaster* (Wolpert, 1978; Gehring, 1985). Therefore, it is very possible that the genetic expression of the male pre-copulatory behavior of the commonly observed wing fluttering is shifted to antennal vibration in blister beetle.

The blister beetle has also been called Spanish fly, and it was used as an aphrodisiac drug in China for many years (Lai, 1976; McCormick and Carrel, 1987). The reason why Chinese once thought that the male blister beetle was a potent remedy for male impotency may have been due to their observing the twisted antennae phenomenon. When we analyzed the content or the yellowish fluid from the joints of our collected beetles' forelegs cantharidin, we observed many peaks in the resulting gas-chromatogram. One of the peaks gave mass spectral data (M/Z intensity(%) 53(15), 70(49), 81(18), 96(94), 100(14), 109(8), and 128(100)) identical to the previously-observed standard cantharidin (Sigma) mass spectrum. Cantharidin

peaks were identified for both males and females with concentrations of  $1287 \pm 199$  and  $448 \pm 72$  ng/ $\mu$ l respectively. Thus, males contained almost three times as much cantharidin as females — a result similar to that reported for *Epicauta occidentalis* (Blodgett *et al.*, 1991). It is thought scientifically that the secretion of cantharidin could be used as a defensive response in other blister beetles; it has also an irritating effect on human skin (Lai, 1976; McCormick and Correl, 1987). These results also confirmed our reasonable exposition that the cantharidin used as an aphrodisiac drug in ancient China may be due to a pre-copulatory behavior of the blister beetles.

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## 臺灣豆莞菁 *Epicauta hirticornis* 交尾前的 觸角纏繞行爲

周 延 鑫      寇 融      蔡 如 秀      賀 孝 雍

本地產的豆莞菁 *Epicauta hirticornis* Haag-Rutenberg 雌雄成蟲在交尾前有一種特別的行為，那就是雄蟲騎在雌蟲背上時，雄蟲的觸角會不斷的擺動，同邊的雄觸角會舞向雌觸角，有時雄觸角會打到雌觸角，因而互相纏繞而形成螺旋狀之構造。右邊的自右向左打，形成右旋，左邊的同理形成左旋。形成右旋或左旋的機率是同等的。假如雌性左邊的觸角不能得到時，則雄性右邊的將和雌性右邊的觸角纏繞，否則，雄性左邊的觸角將和雌性左邊的纏繞。當左右兩邊雌雄觸角都能纏繞時，則此對螺旋觸角還會振動，其振動頻率是 7-12 週 / 秒，極像蛾類交尾時的振翅 (wing fluttering)。莞菁在中國曾被用做壯陽藥物，並且含有莞菁素，故可能與此交尾前之行爲有關。