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REVIEW

Recent Advances in Sex Pheromone Studies on the White-Spotted Longicorn Beetle, *Anoplophora malasiaca*

Midori FUKAYA*

Laboratory of Insect Behavior, National Institute of Agrobiological Sciences
(Tsukuba, Ibaraki 305–8634, Japan)

Abstract

Anoplophora malasiaca (Thomson) (Coleoptera: Cerambycidae) male approached the female, touched her with his antennae or tarsi, and then held and mounted her by touching her elytra and pronotum with his palpae. Similar mating attempts by males were confirmed on a glass dummy treated with the solvent extract of female elytra, indicating the presence of a female sex pheromone perceptible by direct contact. When the extract was chromatographed on silica gel, only the hexane fraction showed a weak activity. The activity was apparently enhanced when polar fractions were subsequently mixed with this fraction. Therefore, the sex pheromone consisted of hydrocarbons and polar compounds that operated synergistically. Among more than 40 hydrocarbons identified by GC-MS analysis, only saturated hydrocarbons induced the mating behavior in males. When 8 major authentic hydrocarbons were mixed together with the polar compounds, the blend induced a series of precopulative behaviors in males. Thus, these hydrocarbons were considered to be the components of the sex pheromone in this beetle. Sexual dimorphism was observed in the hydrocarbon profiles. Some hydrocarbons were common between the sexes while others were sexually specific. A blend of male hydrocarbons and female polar components was still active but the opposite combination was inactive. Therefore, polar female-specific components were critical for revealing the sex pheromonal activity.

Discipline: Insect pest
Additional key words: contact pheromone, hydrocarbons, mating behavior

Introduction—The issue of the "white-spotted longicorn"

The white-spotted longicorn beetle, *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae) feeds on various trees including citrus, apple, pear, willow, plane, white birch, maple, etc. in Japan[17]. This species is an economically serious pest, especially on citrus trees. Adults emerge in early summer and copulate repeatedly. The females lay eggs under the bark on the host trees throughout the summer season. The larvae bore the trunks to feed on the cambium and xylem and the damaged trees eventually die. The tolerable pest density is low, especially for citrus trees. The larvae in the depth of trunks are protected from the direct effect of insecticides. The adults show a high dispersal activity in the citrus grove[1,2]. These are the reasons why it is difficult to control this species by conventional methods.

The genus *Anoplophora* in East Asia contains several species that are similar in both morphological and ecological characters. These similarities have often resulted in taxonomical confusion[18]. *A. malasiaca* had been considered to be distributed also in Taiwan and Mainland China. However, Makihara[19] has recently revealed that this *Anoplophora* species in China is a different species, i.e., *A. chinensis*, and that the actual distribution of *A. malasiaca* should be limited to Japan and Korea.

A similar species, *A. glabripennis,* causes serious damage on poplars that are planted in the arid zone of western China in order to prevent sandy soils from moving[7,22]. Several years ago, *A. glabripennis* was accidentally introduced into North America and it became a serious problem for the United States[6,20]. It was assumed in the USA that *A. glabripennis* was distributed in Japan[6],

*Corresponding author: fax +81–29–838–6205; e-mail fukaya@affrc.go.jp
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based on a collection record performed 90 years ago, although this species is not considered to be distributed in Japan presently[16,18–20,28].

In Europe and in the Mediterranean countries, *A. malasiaca* is one of the most serious insect pests for potential introduction[20,29]. *A. malasiaca* has been occasionally found in *bonsai*, decorative dwarf trees in pots, exported from East Asia to Europe or North America[4,5,25], although there still remain possibilities of confusion in species identification and/or original export area. Host plant species described in the literature are so numerous[17,27] that *A. malasiaca* could well be introduced to various areas. This longicorn beetle might cause problems in international trade if the introduction of the species were to be suspected[16,20]. These circumstances also prompted us to conduct active investigations on the mating behavior and pheromonal communication in this species.

Mating behavior of the white-spotted longicorn beetle

In cerambycid beetles, sex pheromones have been considered to be essential factors in the mating behavior. The female sex pheromone has been detected in all the species in which mating systems were investigated[15]. However, effective distance of the female pheromones varies considerably among the species; in some species, the sex pheromone is perceived only by direct contact of the female with male sensory organs, while it attracts males from the near vicinity or from much longer distances over 1 m in other species.

Males of *A. malasiaca* move between the host trees mainly by flying and in groves by walking[1,2]. Sexual attraction over a long distance is unlikely. Males and females are considered to meet when they wander on the host trees. In laboratory observations, mating frequently took place when both sexes met during wandering (Fig. 1)[10]. Once a male touched a female with his antennae or tarsi, he dashed toward the female to hold her. Then the male licked the female on her back, mounted her, adjusted his body axis to that of the female, bent his abdominal tip over that of the female, and tried to copulate with her (Fig. 1). This successive mating sequence resembles that of *Psacothea hilaris*[8].

The presence of female sex pheromones was demonstrated on the female body surface in *A. malasiaca*. To examine the pheromonal activity, male behavioral responses toward a glass rod coated with female elytrum extract were examined. Males held, mounted and tried to

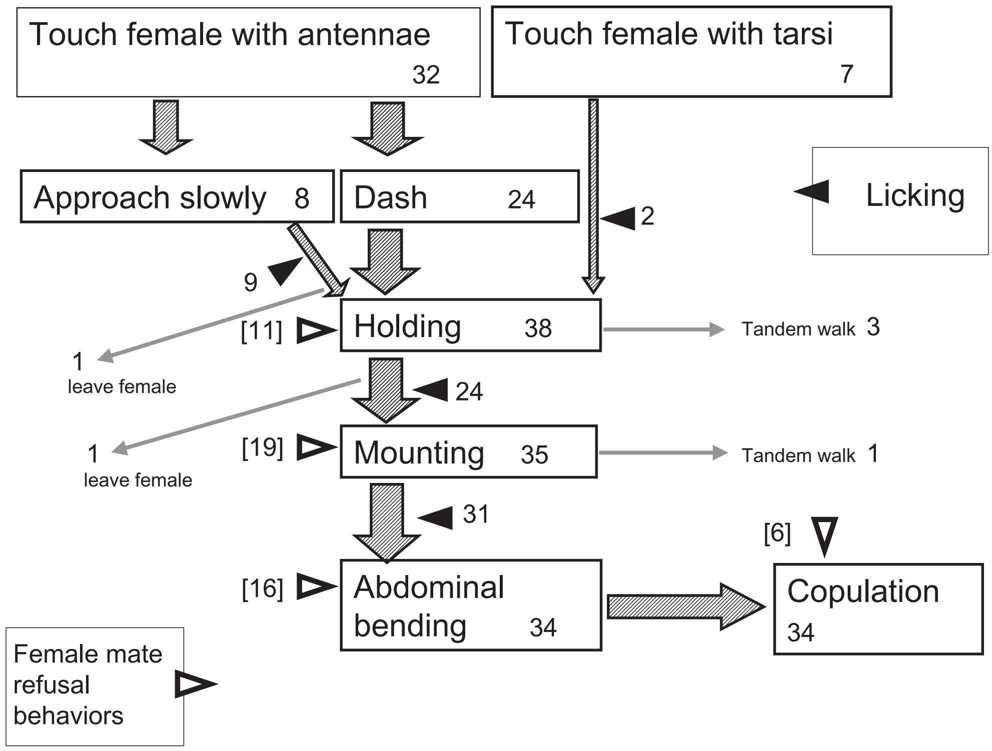

Fig. 1. Mating sequence of *Anoplophora malasiaca* males, and female mate refusal
Each value indicates the number of males exhibiting the behavior in 39 observations. Number in parenthesis also indicates the number of female individuals showing the mate refusal behavior in each phase.

Fig. 2. Male *Anoplophora malasiaca* showing the abdominal bending behavior on a glass dummy coated with a blend of synthetic hydrocarbons and ether fraction of female elytrum extract

copulate with the glass rod in the same way as with a live female (Fig. 2), confirming that the female pheromone induces a series of precopulating behaviors in males[10].

Cuticular hydrocarbons as contact sex pheromone components

The ether extracts of elytra of the female of *A. malasiaca* were applied on a chromatography column of silica gel, and fractions eluted with hexane, 5% and 15% ether in hexane, and ether were obtained. When the single fractions were subjected to behavioral assays, a weak activity was observed only with the hexane fraction and no activity was recorded with the other fractions (Fig. 3). When the hexane fraction was blended with the other polar fractions, the activity apparently increased. These findings indicated that contact sex pheromone of this species consisted of multiple components[11].

GC-MS analyses showed that the hexane fraction contained more than 40 hydrocarbons[11]. Hydrocarbons were further separated into groups of saturated, monoenyl-, dienyl- and trienyl-unsaturated hydrocarbons. The saturated hydrocarbon group showed a high activity and the activity did not increase when this group was blended with the other groups of unsaturated hydrocarbons. The major 8 hydrocarbons were synthesized or purchased for blending at the natural ratio. The hydrocarbon blend induced an abdominal bending behavior in 50% of the males (N = 20) and this value was comparable to that for the natural hexane fraction (60%), when added to the ether fraction. This fact suggested that pheromonally

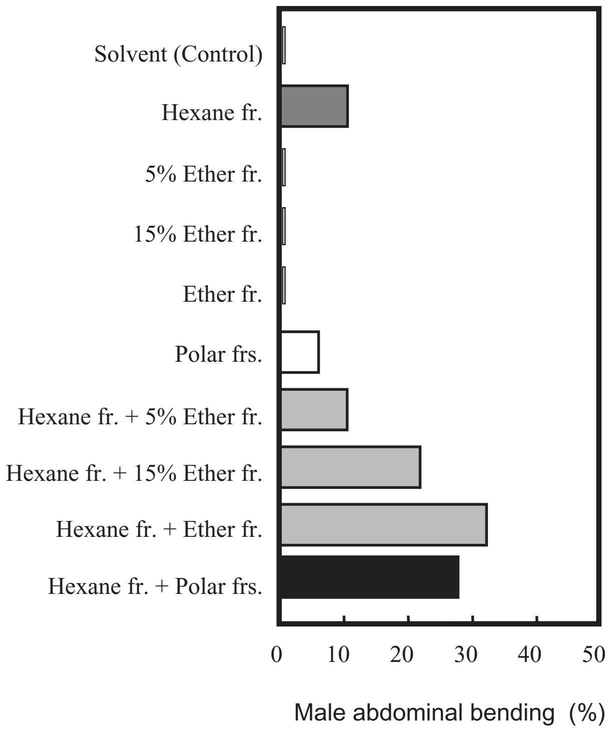

Fig. 3. Behavioral response of male *Anoplophora malasiaca* to crude extract of female elytra and fractions of silica gel column chromatography

Polar frs.: Mixture of 5% and 15% ether in hexane fractions and ether fraction.

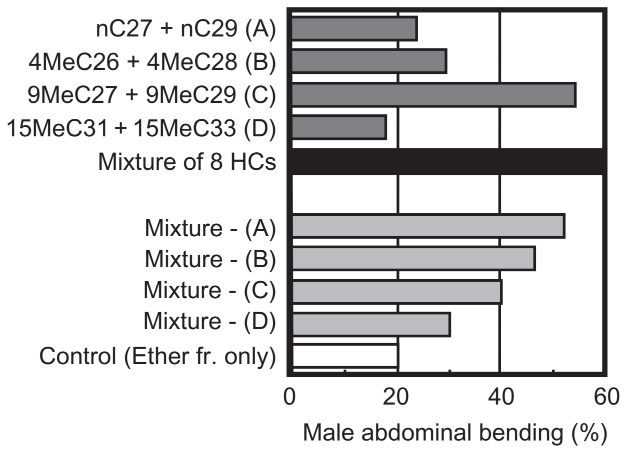

Fig. 4. Behavioral response of male to saturated hydrocarbons (HCs) blended with ether fraction
Dose: 4 female equivalent, N = 30. nC27, heptacosane; nC29, nonacosane; 4MeC26, 4-methylhexacosane; 4MeC28, 4-methyloctacosane; 9MeC27, 9-methylheptacosane; 9MeC29, 9-methylnonacosane; 15MeC31, 15-methylhentriacontane; 15MeC33, 15-methyltritriacontane; mixture-(A) to (D), hydrocarbon mixture lacking A to D.

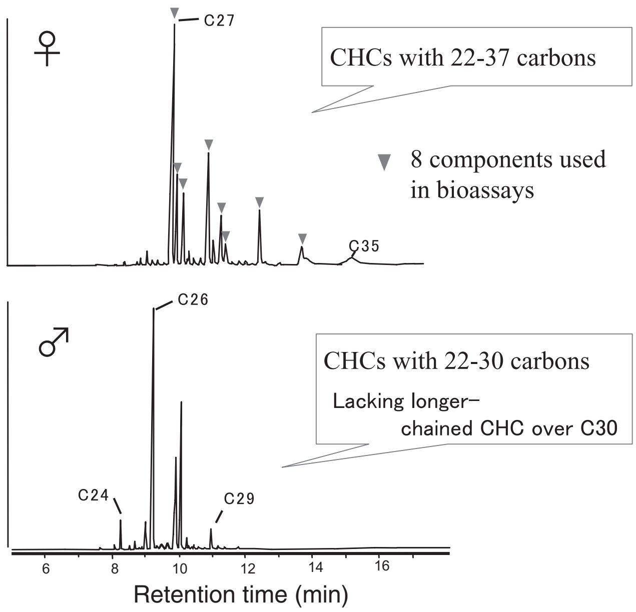

Fig. 5. Gas chromatograms of elytral cuticular hydro carbons (CHCs) from female and male *Anoplophora malasiaca*

active component(s) might be contained in the 8 hydrocarbons.

To determine which component(s) is essential, hydrocarbons were blended with the polar fractions to examine the pheromonal activity (Fig. 4). The blend of hydrocarbons with a methyl branch at the 9-position (9-methylalkanes, hereafter) showed an activity as high as that of the blend of 8 components. However, the lack of 9-methylalkanes (Mixture -(A), in Fig. 4) did not lead to a significant reduction in activity, while the reduction in activity was largest when 15-methylalkanes that did not show an activity in single use were lacking.

In the case of the contact sex pheromone of *A. malasiaca*, although specific component(s) did not seem to be essential for revealing the pheromonal activity, these hydrocarbons appeared to display a compensatory function: the lack of any of the component(s) did not cause any significant reduction in the pheromonal activity (Fig. 4). In the males, the hydrocarbon blend was different from that of the females and the hydrocarbon remained active when blended with the polar fraction of the female extract[3].

Polar components of contact pheromone in *Anoplophora malasiaca*

The contact pheromone of *A. malasiaca* contains polar components in addition to cuticular hydrocarbons, and the mating behavior is induced only when these components are blended. In contrast, polar component(s) also shows a pheromonal activity in *P. hilaris*, in which hydrocarbon blending is not necessary[9]. Our finding in *A. malasiaca,* that the blending of cuticular hydrocarbons and polar components is essential to reveal the pheromonal activity had not been reported in other longicorn beetles (our study is the only one in the world to provide an analysis of the contact pheromone in the longicorn beetle). We detected 2 or more groups of pheromonally active components, and the identification of their chemical structure is in progress (Recently 4 ketone compounds have been identified: Yasui et al., 2003)[23].

Male mate recognition and sexual dimorphism of the cuticular hydrocarbons

Male extract applied to a dummy seldom induced male mating behavior. Is the male or female cuticular hydrocarbon involved in mate recognition in this beetle? We examined the male and female cuticular hydrocarbon profiles and found a significant difference between the sexes (Fig. 5). More than 40 hydrocarbons were identified in the female and male elytrum extracts. Some of them were common between the sexes while others were sexually specific. Female hydrocarbons were characterized by rather long carbon-chains compared with the male ones.

Bioactivity was evaluated for the blends of the hydrocarbon fractions and polar fractions with ordinal and extra-ordinal combinations. A blend of male hydro-

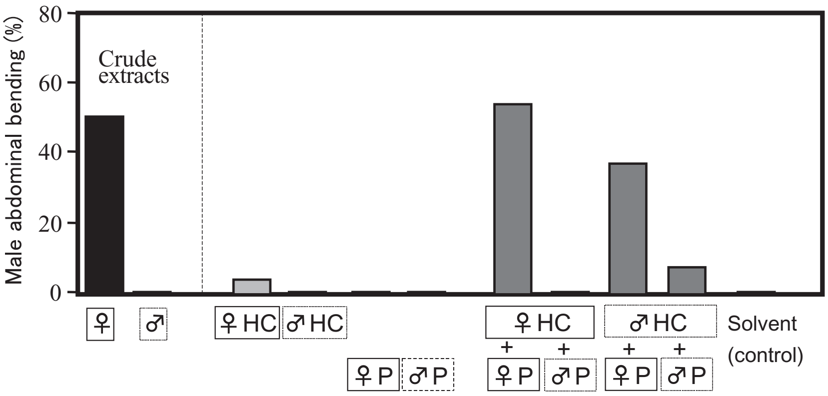

Fig. 6. Male abdominal bending response to cuticular hydrocarbon fraction (hexane fraction) and / or polar fraction (= ether fraction) from female and male ether extracts (1 insect equivalent each, N = 30)
HC, cuticular hydrocarbon fraction; P, polar fraction.

carbon fraction and female polar fraction induced the male mating behavior but the response was slightly weaker than when the crude extract of the female or the blend of hydrocarbon and polar fractions of the females was used (Fig. 6). In contrast, when the male polar fraction was mixed with the female hydrocarbon fraction, male mating response was not induced, indicating that an essential factor that causes significant differences in the male behavior is contained in the polar fractions[3].

Complex information system in mate recognition in *Anoplophora malasiaca*

Cuticular hydrocarbons cover the body surface of arthropods to prevent them from losing water by evaporation. Cuticular hydrocarbons are known to act as semiochemicals in some insects, such as robe beetles and flies[14]. It had been considered that for some species, the composition rate of the hydrocarbons is an important information, while others use one or a few specific hydrocarbon components to recognize their mate. The sex pheromone of *A. malasiaca* is a unique case: (1) 2 (or more) groups of chemical components are needed to explore the sex pheromone activity, and (2) each active group contains more than two components, and moreover each component is complementary to reveal the pheromonal activity.

In the Cerambycidae species, information for a detailed understanding of sexual communication has been very limited. In *A. malasiaca*, more complex aspects of mate recognition than expected will be revealed. Both visual and chemical stimuli are involved in behavioral regulation systems. Contact pheromone is essential for mating recognition in association with visual factors (Fukaya et al., unpublished data).

Analysis of sex pheromones in other *Anoplophora* species

Chemical analysis and identification of behavior regulators are in progress for the East Asian *Anoplophora* species. Existence of a female sex pheromone was confirmed in the citrus longhorned beetle *A. chinensis*[21], although it has not been identified yet. Research projects on *A. glabripennis* are proceeding in China and USA[12,13,24,26]. Recently, it has been reported that 2 male specific substances, 4-heptyloxybutanal and 4-heptyloxybutan-1-ol, acted as stimulatory volatiles in *A. glabripennis*[24,26]. Although both male and female were attracted to a blend of these compounds in a Y-tube olfactometer in the laboratory, field tests conducted in China failed to demonstrate attraction to the 2 compounds[26].

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