



“Double-Trick” Visual and Chemical Mimicry by the Juvenile Orchid Mantis *Hymenopus coronatus* used in Predation of the Oriental Honeybee *Apis cerana*

Authors: Mizuno, Takafumi, Yamaguchi, Susumu, Yamamoto, Ichiro, Yamaoka, Ryohei, and Akino, Toshiharu

Source: Zoological Science, 31(12) : 795-801

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zs140126>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

“Double-trick” Visual and Chemical Mimicry by the Juvenile Orchid Mantis *Hymenopus coronatus* Used in Predation of the Oriental Honeybee *Apis cerana*

Takafumi Mizuno^{1*}, Susumu Yamaguchi², Ichiro Yamamoto³,
Ryohei Yamaoka⁴, and Toshiharu Akino¹

¹Applied Entomology Laboratory, Center for Bioresources Field Science, Kyoto Institute of Technology, Saga-ippongi-cho 1, Kyoto 616-8354, Japan

²Wakamiko 4495-141, Sudama-cho, Hokuto-shi, Yamanashi 408-0112, Japan

³Win-K Corporation, 4-1-24 No.1 2FC+ Jinguumae, Shibuya-ku, Tokyo 150-0001, Japan

⁴Kami-ikedada-cho 55-3, Kitashirakawa, Kyoto 606-8287, Japan

It has long been hypothesized that the flower-like appearance of the juvenile orchid mantis is used as visual camouflage to capture flower-visiting insects, although it is doubtful whether such morphological resemblance alone could increase their success in hunting. We confirmed that juvenile female orchid mantises often succeed in capturing oriental honeybees, while adult females often fail. Since most of the honeybees approached the juveniles from the front, we hypothesized that juvenile orchid mantises might attract honeybees by emitting some volatile chemical cues. Gas chromatography-mass spectrometry analyses revealed that the mantises' mandibular adducts contained 3-hydroxyoctanoic acid (3HOA) and 10-hydroxy-(E)-2-decenoic acid (10HDA), both of which are also features of the pheromone communication of the oriental honeybee. We also successfully detected 3HOA emitted in the head space air only at the time when the juvenile mantises were attempting to capture their prey. Field bioassay showed that the Oriental Honeybee predominantly preferred to visit dummies impregnated with a mixture of the appropriate amounts and ratios of 3HOA and 10HDA. We therefore conclude that the juvenile mantises utilize these as allelochemicals to trick and attract oriental honeybees.

Key words: orchid mantis, *Hymenopus coronatus*, oriental honeybee, *Apis cerana*, aggressive mimicry, chemical mimicry

INTRODUCTION

Flower detection and location by flower-visiting insects depend on visual and olfactory signals from the plant. Entomophilous plants, for example, often appeal to pollinator insects by the shapes and patterns of their petals, UV-positive brightly colored flowers, and scents, all of which such features successfully guide the pollinators from a distance (Barth, 1991; Chittka and Menzel, 1992; Kevan and Baker, 1983; Lunau, 1996; Dötterl and Jürgens, 2005; Foster et al., 2014). These plants occasionally present floral signposts, including nectar guide and pollen guide, to lead pollinators to them in order to guarantee their rewards. Such floral signposts of entomophilous plants are often visible only under longer wavelength ultraviolet rays, which is presumably because various pollinator insects have UV-sensitive vision.

Prey detection and location by predatory animals also depend on visual, olfactory, and acoustic signals from their prey, and this has resulted in the selection of adaptations by prey species that help avoid such predatory animals; one of

the general patterns being collectively known as camouflage. Camouflage includes crypsis and masquerade (Stevens and Merilaita, 2009). Crypsis is the ability of an animal to avoid observation or detection by other animals, while masquerade is used to hinder recognition by other animals. These concealment tactics are effective not only for antipredation but also predation. When a predator uses such a concealment camouflage tactic to capture prey, it is referred to as aggressive mimicry. Such aggressive mimicry is reported in crab spiders (Heiling et al., 2003, 2005), stick mantises, dead-leaf mantises (Edmund, 1972; Evans and Schmidt, 1990; Stoddard, 2012) and flower mantises (Wickler, 1968; Owen, 1980).

The orchid mantis, *Hymenopus coronatus*, which inhabits Southeast Asia, has a unique appearance resembling the flowers of the sympatric orchid *Melastoma polyanthum* (Wickler, 1968; Gullan and Cranston, 2010). The juveniles, in particular, resemble a flower, as their abdomens are bent upwards, while adults do not bear as much resemblance to flowers, because their wings prevent them from being able to bend their abdomens in the same way that juveniles can. It is commonly believed that orchid mantises hide themselves among flowers in order to trick and capture flower-visiting insects (Wickler, 1968; Owen, 1980). This idea was recently

* Corresponding author. Tel. : +81-90-6757-1660;
Fax : +81-75-861-0790;
E-mail: takafumi.takafumizuno@gmail.com
doi:10.2108/zs140126

supported by comparing the spectral reflectance between orchid mantis and sympatric flowers including 13 species from 10 genera e.g., *Melastoma*, *Medinilla*, and *Tabernaemontana*. (O'Hanlon et al., 2014b). Since both juvenile and adult mantis similarly absorbed the ultraviolet rays, they were theoretically indistinguishable for hymenopteran pollinators, which have UV vision (O'Hanlon et al., 2013, 2014a, 2014b). Although orchid mantis do not imitate any particular flower species, the spectral reflectance of their bodies contributes to their floral mimicry for the purpose of deceiving hymenopteran pollinators (O'Hanlon et al., 2014a, 2014b). Such ecological and visual physiological approaches were valuable for evaluating the visual resemblance between the orchid mantis and flowers. But it remains doubtful whether this visual resemblance alone could contribute to their aggressive mimicry.

To confirm the speculation that orchid mantis hide themselves among flowers in order to capture various flower-visiting insects (Wickler, 1968; Owen, 1980), we first focused on the hiding and ambushing sites of the orchid mantis in the field. This field research was conducted in Thailand, Malaysia, and Indonesia to record hiding and ambush sites of orchid mantis, and also to record the prey that they actually captured. Since the prey species of the female juvenile orchid mantis was apparently biased, we further investigated the possibility that chemical signals could be involved in the chemical aggressive mimicry of the orchid mantis.

MATERIALS AND METHODS

In 2013, 40 juvenile female orchid mantis collected in Indonesia were transferred to Japan, and individually kept in plastic cups (11 cm diam., 10 cm height) at ca. 25°C with 14L:10D photoperiods in an insectary of the Center for Bioresource Field Science (CBFS) at the Kyoto Institute of Technology (KIT) in Kyoto, Japan. Younger juveniles, estimated to be from the 2nd to 3rd instars, were fed with European house crickets, *Acheta domestica*, every day, and older juveniles, which were estimated to be from the 4th to the last instars, were fed them every other day. Worker honeybees of *Apis cerana japonica*, one of the domestic subspecies of *Apis cerana*, were captured in an agricultural field of CBFS at KIT and used for further chemical analysis. A behavioral bioassay was conducted in the same field of CBFS using forager workers of the honeybee.

We observed the foraging habits of 28 orchid mantis we found at three different sites (Chiang Mai in Thailand, the Cameron Highlands in Malaysia, and East Java in Indonesia), and recorded each developmental stage (juvenile or adult), hiding and ambush site, and prey that they captured and attempted to capture. During the daytime orchid mantis were often at rest in the shade without attempting to hunt, continuous observations were conducted both in the morning and evening. When we found mantis in the field, we started observation of their foraging habits directly with the naked eye, while simultaneously recording them by two types of video recording camera, the Bee-CAM (NHK Science & Technical Research Laboratories) and a high-speed camera (FASTCAM BC2HD; Photron) in 1000 fps. The Bee-CAM was utilized to compare UV absorption between the juvenile orchid mantis and sympatric flowers. Because this camera is sensitive to blue, green, and UV wavelengths, but insensitive to red, any red color in the Bee-CAM photos indicates the reflection of UV rays.

A total of nine female juvenile orchid mantis were dissected to separate their upper bodies into three parts: mandibles, with their appendages; the rest of the head capsules; and stickles. Each body part was then immersed in an appropriate volume of dichloromethane (Wako Pure Chemical Industries Ltd.) for 30 min. Whole head parts were separated from three adult orchid mantis, and each was crushed and also immersed in the appropriate volume of dichloromethane for 30 min. In the same manner, we also made an extract from each pair of the mandibular glands dissected from three workers of the Japanese oriental honeybee, *Apis cerana japonica*.

romethane (Wako Pure Chemical Industries Ltd.) for 30 min. Whole head parts were separated from three adult orchid mantis, and each was crushed and also immersed in the appropriate volume of dichloromethane for 30 min. In the same manner, we also made an extract from each pair of the mandibular glands dissected from three workers of the Japanese oriental honeybee, *Apis cerana japonica*.

Volatile chemicals emitted by the orchid mantis were collected for 20 min. with a glass-bead trap made from a Pasteur pipette packed with ca. 2 g of glass beads (1 mm diam.). One end of the pipette was connected to a glass funnel (30 mm diam.), and the opposite end was connected to an aspirator by means of a plastic tube. The funnel was faced towards the orchid mantis at a distance of about 1 cm to collect volatile chemicals secreted by it in two different conditions: when a juvenile female orchid mantis was attempting to capture a dead honeybee swinging on a string in front of it and alternatively when it was merely sitting-and-waiting without looking at any prey item. Further, as a control, we also collected blank-odor without using the mantis. Each trial was repeated eight times using different orchid mantis individuals.

The glass-bead funnel trap was also applied in order to collect volatile chemicals from filter paper impregnated with authentic chemicals: 100 µg of 3-hydroxyoctanoic acid (3HOA Wako Pure Chemical Ind. Ltd.) and 10 µg of 10-hydroxy-(E)-2-decenoic acid (10HDA Nagara Science Ind. Ltd.). Each trial was conducted for 20 min., and separately repeated three times. As a control, blank-odor was also collected from the intact filter paper impregnated with only the solvent, dichloromethane. All the trapped chemicals were then eluted with 3 ml of dichloromethane, and analyzed after concentration.

TMS adducts were obtained by reacting 10 µL of each concentrated extract sample with 2 µL of BSTFA; N,O-bis(trimethylsilyl)trifluoroacetamide (Tokyo Chemical Ind. Ltd.) at the injection port of a gas chromatograph. Methyl esters of the hydroxyl carboxylic acids were obtained by reacting 20 µL of concentrated volatile secretion samples solved in 500 µL of methanol-benzene (2:8 v/v) with 50 µL of trimethylsilyldiazomethane (Tokyo Chemical Ind. Ltd.) for 30 min at room temperature. Authentic 3HOA and 10HDA was derivatized with BSTFA and trimethylsilyldiazomethane, respectively, in the same manner, and 50 ng of the respective TMS adducts were analyzed as standard to quantify the corresponding compounds in the secretion samples.

GC/MS analyses were performed on a Shimadzu GC-MS QP5000 equipped with GC-17A and an apolar capillary column, DB-1HT (J&W, 15 m length, 0.25 mm in diam., 0.10 µm film thickness). EI-mass spectrum was obtained at 70 eV. Injection was made at the splitless mode for 1 min at 300°C, and the interface was also kept at 300°C. The column oven temperature was kept at 40°C for 5 min, programmed to 300°C at 10°C/min, and then kept at the final temperature for 10 min. Helium was used as the carrier gas at the column head pressure of 40 kPa. Volatile secretion samples were analyzed by the selecting ion monitoring (SIM) mode. As diagnostic fragment ions for the SIM mode, we selected five diagnostic ions for 3HOA. The relative intensities were, respectively, *m/z* 71(47.8%), 74(42.5%), 83(12.2%), 103(82.2%) and 125(4.76%) and the six diagnostic ions for the 10HDA were *m/z* 81(57.3%), 87(43.2%), 113(23.0%), 124(16.7%), 138(3.97%) and 168(7.62%) respectively.

To assess the effect of 3HOA and 10HDA on the honeybee's flower-visiting behavior, we conducted two choice bioassays in a plot where Japanese pumpkins *Cucurbita moschata* were flowering, and where Japanese oriental honeybees occasionally foraged. Since pumpkin flowers are yellow, we arranged a piece of yellow paper closely resembling the flower color, three centimeters square, with a five millimeter filter paper attached to the center as a visual mark for the honeybees. We fastened the paper to green poles and stuck two poles into the ground 20 centimeters away each from a pumpkin flower. A test sample was treated on one of the filter

papers attached to the yellow paper and solvent dichloromethane was treated on the other to serve as a control. We tested four kinds of samples: the mixtures of 3HOA and 10HDA (100 and 10 μg , and 100 and 1 μg respectively), and a single sample of 3HOA (100 μg) and 10HDA (10 μg). The number of honeybees orienting the paper of each sample and control within five centimeters was counted. Each observation was continued for 10 minutes and eight replicates on each of the test samples were tested one by one.

We tested for the differences between adults and juveniles in hiding and ambushing sites with Fisher's exact test and in capturing prey items with a Chi-square test. Additionally, we conducted residual analysis for components of the prey items. The difference in the amount of secretion was tested using the Steel-Dwass test, and the difference in the honeybees' preferences between the artificial chemical specimen and the control was tested for with a binomial test.

RESULTS

Our field observation of the orchid mantises resulted in being inclined towards female adult and juvenile older than the third instar, because of the difficulty of finding juveniles younger than the second instar and males in the field. Hiding and ambushing sites are significantly different between the juvenile and adult orchid mantises (Table 1 Fisher's exact test $P < 0.01$). A total of 24 juveniles and four female adults were found in the field. While all the juveniles were found on leaves, three adults were on flowers (two on papaya, *Carica papaya*, and one on an uncertain species of Liliaceae), and the fourth was found copulating on a leaf.

Prey items are also significantly different between of the juveniles and adults (Table 1 Chi-square test $P < 0.01$). While the adult Orchid Mantises attempted to capture flower-visiting lepidopterans and hymenopterans, the juveniles fairly frequently succeeded in capturing the domestic oriental honeybee, *Apis cerana cerana*. The number of lepidopterans captured by adults was significantly greater than that captured by juveniles (residual analysis $P < 0.01$), while the number of hymenopterans that the juveniles attempted to capture was significantly larger than that which the adults attempted to capture (residual analysis $P < 0.01$). The success rate for capture was higher in juveniles (90.9%, 33 trials) than in adults (62.5%, eight trials). The juvenile successfully captured the honeybees flying to visit them, but the adults often failed to capture flying butterflies. The adults observed tried to seize flying butterflies at least six times, so their hunting success rate was 50%. On two separate occasions, we observed adult mantises capturing hymenopterans that had landed on flowers. But one adult mantis failed to catch the prey, a large wasp. Thus, the successful capture rate of flying insects was significantly higher in the juveniles than the adults (Chi-square test $P < 0.05$). Female juveniles presumed to

be older than third instar and female adults are shown in this study, because we could find few first or second instars or males in the field.

Seen through a Bee-CAM, the juvenile mantis looked blue and the surrounding leaves looked red (Fig. 1A). This means that the mantis absorbed the UV rays, while the leaves reflected them. It was also confirmed that flowers looked blue and the surrounding leaves looked green (Fig. 1B). This means that the flowers also absorbed the UV rays, but the leaves didn't significantly absorb or reflect UV. In our observations, no background foliage absorbed UV. Therefore, juvenile orchid mantises and UV-absorbing flowers always have a high contrast with the surrounding foliage. When an image of an adult orchid mantis located on a papaya flower was taken by the Bee-CAM, both the mantis and the flower appeared blue. Thus, it is clear that they had both absorbed UV, and, consequently, there was a low contrast between the adult and the background flowers.

The high-speed camera clarified the process of

Table 1. Ambush sites and prey insects of juvenile and adult orchid mantises in the field.

Orchid Mantis	Ambushing sites		Prey insects			
	Flower	Leaf	hymenopteran	dipteran	lepidopteran	hemipteran
Juvenile	0	24	24 (3)	5 (0)	0 (0)	1 (0)
Adult	3	1	1 (1)	0 (0)	3 (3)	0 (0)

The numbers in parenthesis show the number of insects that mantises tried and failed to capture.

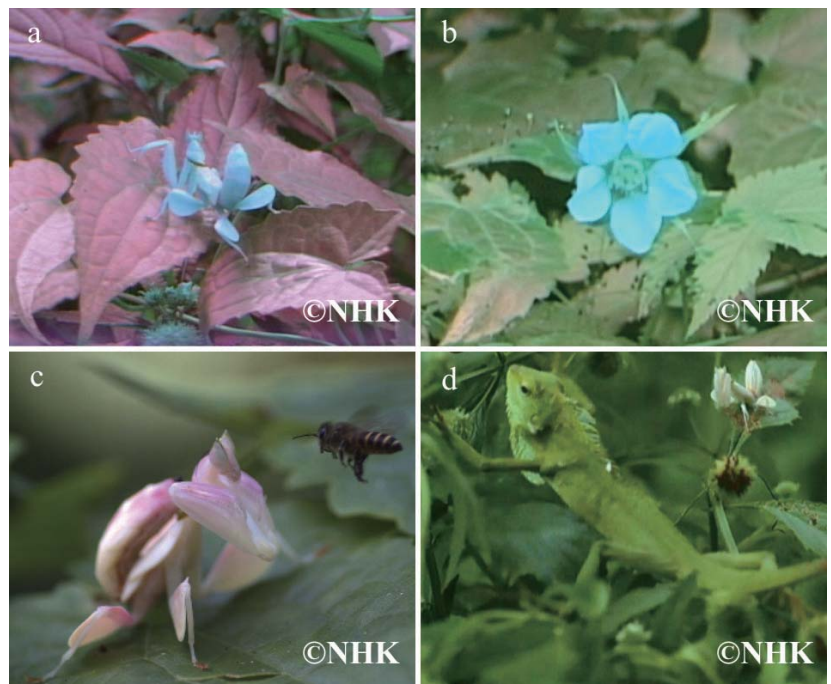


Fig. 1. Juvenile orchid mantis and sympatric flowers seen through the Bee-CAM and High-speed camera. (A) UV-sensitive Bee-CAM photo of UV-absorbing juvenile orchid mantis colored blue and UV-reflecting foliage colored red (B) Bee-CAM photo of a UV-absorbing flower (*Rubus* sp.) in the habitat of the orchid mantis (C) An oriental honeybee located in front of a juvenile orchid mantis and trying to land on it (D) A lizard passing by an orchid mantis without showing any interest in it. These photographs were provided by NHK, supporting our field work in Southeastern Asian countries.

approach by oriental honeybees, *Apis cerana cerana*, to female juvenile orchid mantis (Fig. 1C). The honeybee prowled nearby the orchid mantis and then approached to the front of the mantis head on, stretching its hind legs and hovering within the range of the stickles. In other words, the honeybee tried to land on the mantis just as it would on a flower. Such approach behavior was repeatedly observed in the other pairs of honeybees and orchid mantis.

Only on one occasion were we able to observe the interaction between a juvenile orchid mantis and a predator, when a lizard and a juvenile mantis encountered one another. The orchid mantis, situated on a leaf, was conspicuous but completely motionless, and although the lizard once stood in front of it, only about 30 centimeters away, it eventually passed it by with indifference (Fig. 1D).

The mandibles and their appendages of the female juvenile orchid mantis and the mandibular glands of the oriental honeybee, *Apis cerana japonica*, possessed the same chemicals. The Kovats Retention index and mass spectra of the trimethylsilylated components (I^{RP} : 1487 and I^{RP} : 1858) of the mantis and honeybee corresponded with those of two authentic 3HOA and 10HDA, respectively (Fig. 2).

The amount and the ratio of the two chemicals of juvenile orchid mantis varied widely between individuals (Fig. 3). Some juveniles possessed 2226.9 ng 3HOA and 22.2 ng 10HDA, while some possessed none of these chemicals. However, none of the adult orchid mantis examined possessed both of them.

GC-MS SIM mode analysis of volatile secretion samples showed a series of ions with identical retention time and relative intensities as authentic 3HOA (Fig. 4). Significantly more 3HOA was detected in samples collected from hunting mode mantis than from the control (Fig. 5 $P < 0.05$, Steel-Dwass test). There were no significant differences, however, between samples collected from sitting-and-waiting mode mantis and the control. Quantitative GC-MS analyses detected total of 7.2, 8.9, and 16.7 ng of 3HOA from three replicates, respectively, in 20 min collection from the filter paper each treated with a mixture of 100 μg 3HOA and 10 μg 10HDA. These values were more or less equal to the amount of 3HOA secreted by the hunting mode mantis. No 10HDA was detected in any of these test samples.

The sample with an applied mixture of 100 μg 3HOA and 10 μg of 10HDA had a significantly stronger attractive effect than the control (Fig. 6 $P < 0.01$ binomial test). Of the 24 times that honeybees approached, 19 honeybees oriented towards the sample. Also, the samples with an applied mixture of 100 μg 3HOA and 1 μg of 10HDA oriented 10 honeybees of 13 (Fig. 6 $P < 0.05$ binomial test). By contrast, the papers with an individual chemical applied did not have a significantly stronger attractive effect than the control.

DISCUSSION

The orchid mantis bears a close resemblance to a real flower, not only when seen through human eyes but also through an insect's. As shown in Bee-CAM photos, both the juvenile and adult orchid mantis, as well as flowers, absorbed UV rays, which suggests it would be difficult for UV-visible flower-visiting insects such as oriental honeybees to distinguish the orchid mantis from sympatric flowers (Fig.

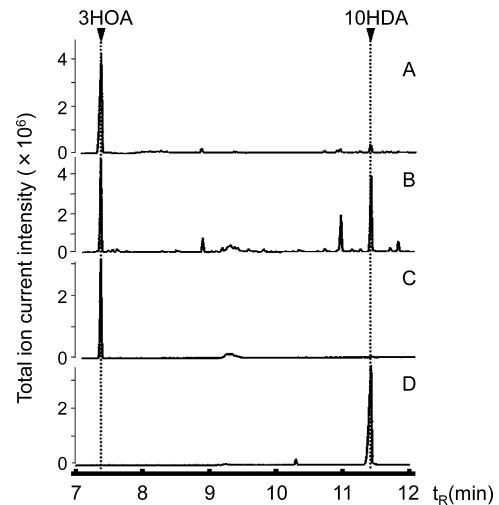


Fig. 2. Comparison of sample extracts and synthetic samples. Total ion chromatograms shown are TMS derivatives of (A) extract of mandibles and their appendages from the juvenile orchid mantis, (B) extract of mandibular glands from the oriental honeybee, (C) pure 3-hydroxy octanoic acid (3HOA) and (D) pure 10-hydroxy-(E)-2-decanoic acid (10HDA).

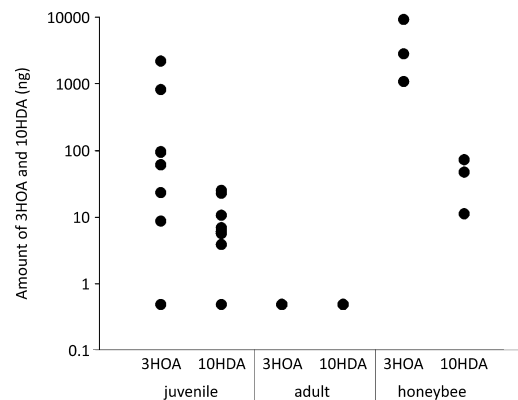


Fig. 3. Quantitative comparison of 3HOA and 10HDA among adult and juvenile orchid mantis and oriental honeybees. Amount of these compounds from each individual was common log-transformed after adding 0.5 (Yamamura, 1999).

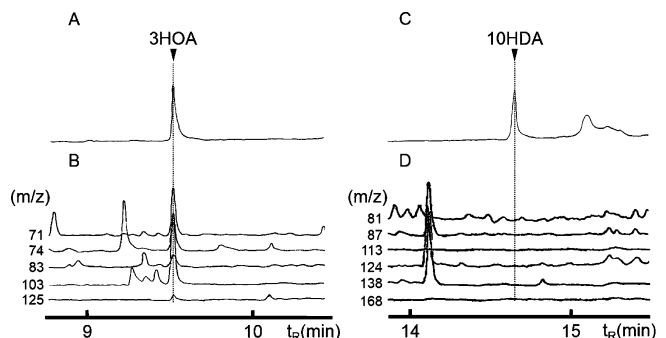


Fig. 4. SIM chromatograms of secreted chemicals and authentic samples. (A) derivatized authentic 3HOA (B) derivatized secretion sample shown by the diagnostic ions at m/z 71, 74, 83, 103 and 125 (C) derivatized authentic 10HDA (D) derivatized secretion sample shown by the diagnostic ions at m/z 81, 87, 113, 124, 138 and 168.

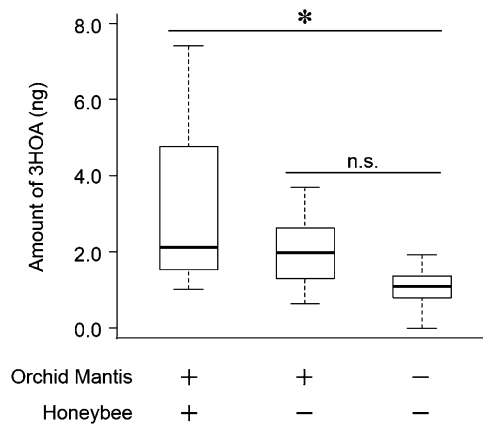


Fig. 5. Amount of 3HOA secreted by orchid mantis among two treatments and a control. Treatments are (from left): orchid mantis attempting to capture a dead honeybee swinging on a string in front of it; sitting-and-waiting orchid mantis with no prey, and a control without an orchid mantis. The numbers on the vertical axis show the amount of secreted 3HOA detected by GC/MS. The Steel-Dwass test was used for statistical analysis; * $P < 0.05$.

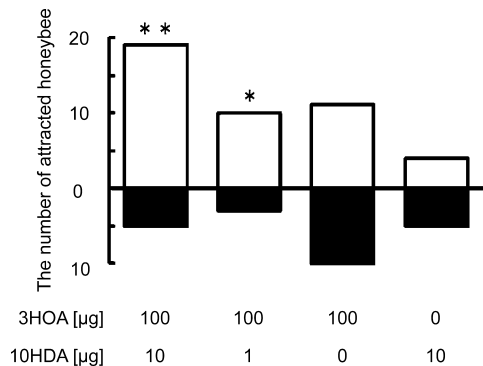


Fig. 6. Attracting effect of hydroxyl fatty acids, 3HOA and 10HDA on oriental honeybees *Apis cerana japonica*. The effect of 3HOA and 10HDA on the approach frequency of the oriental honeybee was evaluated by two-choice tests with a paper disc treated by test samples or solvent as a control. The black and white columns indicate the number of honeybees approaching the visual mark with test samples and with the solvent control, respectively. The numbers on the vertical axis show the number of attracted honeybees to either visual mark. The significance of the results relative to controls was assessed using binominal analyses; * $P < 0.05$, ** $P < 0.01$.

1A, B). This is likely to help juvenile orchid mantis capture prey; although their prey items are obviously biased towards the oriental honeybee (Table 1). This suggested that the juvenile mantes could secrete certain chemicals to deceive and attract the oriental honeybee effectively. Our hypothesis was clearly supported by chemical analyses of the juvenile mantes, which contained 3HOA and 10HDA from organs connected to the mandibles (Fig. 2). Both these compounds are considered to be involved in the pheromone communication of the oriental honeybee. We also confirmed that 3HOA was secreted into the air only when the mantes attempted to hunt (Figs. 4, 5), and, further, that the two compounds actually guided the foraging honeybees to approach when they were impregnated on paper discs in our field bioassay (Fig. 6). These data strongly suggest that the visually-

camouflaged predator, the orchid mantis, uses additional aggressive chemical mimicry to capture the oriental honeybee. To the best of our knowledge, this is the first report of double-trick mimicry by predatory animals via visual and olfactory cues used to deceive their prey.

As observed in Bee-CAM photos, both juvenile and adult orchid mantes and sympatric flowers absorb UV rays, and their colors, including their UV range, were extremely similar. Recent studies (O'Hanlon et al., 2013, 2014a, 2014b) successfully revealed such spectral resemblance, including UV range, between the orchid mantes and flowers, and difficulty for the honeybees to distinguish the color of the orchid mantis from that of some sympatric flowers. We further confirmed the high contrast between the UV-absorbing juvenile orchid mantes and UV-reflecting foliage. It is likely that such high contrast in UV with the background would serve as an attractant guide for the flower-visiting insects. In general, a high visual contrast with the background often influences the foraging behavior of flower-visiting insects (Kinoshita et al., 2011; Spaethe et al., 2001). Certain flowers present such high contrast with the background by partially UV-absorbing petals (e.g., *Mimulus guttatus*, Rae and Vamosi, 2013) in order to attract UV-visible pollinators. According to Heiling et al. (2003, 2005), certain crab spiders capture such UV-visible pollinators by deceiving them with their UV-absorbing bodies.

However, the prey items of the juvenile orchid mantes, which tended towards honeybees, suggest that their predation strategy may rely not only on visual camouflage but also additional tactics, e.g., aggressive chemical mimicry. A famous example of aggressive chemical mimicry is bolas spiders, which capture specific species of male moths by emitting female moth sex pheromones (Eberhard, 1977; Stowe et al., 1987; Gemeno et al., 2000; Haynes et al., 2002). Chemical mimicry is a common and effective tactic to control the behavior of nocturnal insects, including moths, as well as diurnal insects including bees. Some varieties of orchids, for example, imitate the appearance of a bee, and, furthermore, utilize sex pheromones to attract male diurnal bees with both visual and olfactory stimuli (Spaethe et al., 2007, 2010; Streinzer et al., 2009; Rakosy et al., 2012). Such combined visual and olfactory mimicry is probably effective in deceiving the diurnal bees.

Because of the species specificity in the prey items of the juvenile orchid mantes throughout our field study, we hypothesized that the mantes would imitate the semiochemicals that were involved in the pheromone communication of the oriental honeybee. Our hypothesis was supported by the finding that two hydroxy fatty acids, 3HOA and 10HDA, were stored in the mandible appendages of the juvenile orchid mantes, as well as in the mandibular gland of *Apis cerana japonica*, a subspecies of oriental honeybee. Although the ethological function of these two hydroxy fatty acids in pheromone communication has not been determined, oriental honeybee workers do possess these two compounds (e.g., Indonesian *Apcis cerana cerana*, Keeling et al., 2001). It appears likely that these compounds serve as a sort of aggregation signal (Matsuyama, personal communication). Similar chemical mimicry of the oriental honeybee pheromone is also reported in the oriental orchid, *Cymbidium floribundum* (Sugahara et al., 2013). The orchid flower

attracts swarms of the oriental honeybee, not only forager workers but also the drones (male bees), by emitting two hydroxy-fatty acids, 3HOA and 10HDA as a mixture. It is likely that individual forager and worker would serve as pollinator (Sasaki et al., 1991), but ecological implication of swarm attracted by oriental orchid remains obscure. This oriental orchid is famous to attracts many subspecies of *Apis cerana*, which is widely distributed in Southeast Asia, including *Apis cerana cerana* and *Apis cerana japonica*, (Sugahara et al., 2006). It would be, therefore, very effective to imitate such hydroxy fatty acids to deceive the oriental honeybee in these areas. Concerning the secretion of these two compounds by the juvenile orchid mantis, our analyses confirmed that the amount of 3HOA in the air significantly increased only when the juvenile moved its head while chasing the victim and stretched its spiked forelegs to capture it. Although 10HDA was not detected in the air in any cases we tested, it was because the amount of the compound that the juvenile orchid mantis possessed was too small to be detected as a volatile secretion. Since these two compounds were contained in the mandible appendages of the juvenile orchid mantis, they are presumably emitted together at once, when the juveniles intend to capture the victim. In contrast, the adult orchid mantis possessed no 3HOA or 10HDA, and they managed to capture the honeybees less often than the juveniles. These facts also support our hypothesis on aggressive chemical mimicry by the juvenile orchid mantis.

Although the absolute amounts of 3HOA and 10HDA that were emitted by one juvenile orchid mantis were apparently smaller than those from the oriental orchid flower, the emitted substances were sufficient to attract one oriental honeybee forager to the source of the odor. Flying forager honeybees approached filter paper significantly more often when it was impregnated with a mixture of both 3HOA and 10HDA (100 µg:10 µg or 100 µg:1 µg), but not when impregnated with only 3HOA or 10HDA (Fig. 6). From the filter paper impregnated with 100 µg of 3HOA and 10 µg of 10HDA, it was possible to collect and detect 3HOA at the same level as that collected from the hunting mantis. This suggests that the chemicals that juvenile orchid mantis secrete are of sufficient quantity to deceive solitary foragers of the oriental honeybee.

In general, prey-capture by praying mantis is helped by their acute sense of vision, and their use of various kinds of visual camouflage to conceal themselves (Edmund, 1972; Evans and Schmidt, 1990; Stoddard, 2012; Wickler, 1968; Owen, 1980). Their way of walking and swaying movements are also effective in preventing them being noticed by prey animals, especially in the wind (Watanabe and Yano, 2013). While the effects of such visual stimuli on their foraging have been well studied, there are few reports on chemical tactics of the praying mantis, except for intraspecific sexual communication (Hurd et al., 2004; Maxwell et al., 2010; Perez, 2005). Our study is, therefore, the first report on chemical tactics of the praying mantis, which also employs visual camouflage, to lure prey animals using semiochemicals.

Since the Orchid Mantis utilizes the pheromone-mimic chemicals to capture the Oriental Honeybee, this is categorized as aggressive chemical mimicry. Aggressive chemical mimicry is also reported in bolus spiders that deceive noc-

turnal male moths by imitating the respective female moths' sex pheromone (Eberhard, 1977; Stowe et al., 1987; Gemeno et al., 2000; Haynes et al., 2002). Although those nocturnal moths mainly rely on the chemical stimuli to locate females, diurnal honeybees rely on not only chemical but also visual stimuli to visit flowers (Spaethe et al., 2007, 2010; Streinzer et al., 2009; Dötterl and Vereecken, 2010; Milet-Pinheiro et al., 2012; Rakosy et al., 2012). It is likely that the Orchid Mantis, especially the juveniles, might have acquired chemical tactics to capture such flower-visiting insects in addition to its visual mimicry.

In other words, the orchid mantis *H. coronatus* uses a "double-trick" as observed for caterpillars of *Biston robustum* (Akino et al., 2004). Although their double-trick is based on both visual and chemical mimicry, the aims of each species' strategy differ. That of the orchid mantis is to become visually conspicuous and to lure prey items chemically, while that of the caterpillars is to become both visually and chemically cryptic enough to avoid attacks by avian predators and carnivorous ants. Few reports have documented such multiple-trick tactics that deceive other animals, but this does not necessarily indicate that this phenomenon is observed in only a few animals. Visual mimicry is such an impressive phenomenon that it may have kept researchers from exploring additional, less obvious tactics, such as chemical mimicry. We thus hypothesize that multiple-trick mimicry may have evolved in various animals more commonly than we might assume.

ACKNOWLEDGMENTS

We thank Dr. T. Ichioka of Kyoto University for identification of the oriental honeybee, H. Egawa of NHK Enterprises Inc. for providing photos and videotapes of the orchid mantis, S. Tanaka of the National Institute of Agrobiological Sciences for editing our revised English and J. Ryder for proofreading the English of the manuscript. This study is partially supported by Kyoto Institute of Technology-Venture Laboratory (KIT-VL) training grants 2013.

REFERENCES

- Akino T, Nakamura K, Wakamura S (2004) Diet-induced chemical phytomimesis by twig-like caterpillars of *Biston robustum* Butler (Lepidoptera: Geometridae). *Chemoecology* 14: 165–174
- Barth FG (1991) *Insects and Flowers. The Biology of a Partnership.* Princeton University Press
- Chen DM, Goldsmith TH (1986) Four spectral classes of cone in the retinas of birds. *J Comp Physiol A* 159: 473–479
- Chen DM, Collins JS, Goldsmith TH (1984) The ultraviolet receptor of bird retinas. *Science* 225: 337–340
- Chittka L, Menzel R (1992) The evolutionary adaptation of flower colours and the insect pollinators' colour vision. *J Comp Physiol A* 171: 171–181
- Dötterl S, Jürgens A (2005) Spatial fragrance patterns in flowers of *Silene latifolia*: Lilac compounds as olfactory nectar guides? *PL Syst Evol* 225: 99–109
- Dötterl S, Vereecken NJ (2010) The chemical ecology and evolution of bee-flower interactions: a review and perspectives *Can J Zool* 88: 668–697
- Eberhard WG (1977) Aggressive chemicalmimicry by a bolas spider. *Science* 198: 1173–1175
- Edmunds M (1972) Defensive behaviour in Ghanaian praying mantids. *Zool J Linn Soc-Lond* 51: 1–32
- Evans DL, Schmidt JO (1990) *The Evolution of Cryptic Coloration.* State University of New York Press
- Fleishman LJ, Loew ER, Leal M (1993) Ultraviolet vision in lizards.

- Nature 365: 397
- Foster JJ, Sharkey CR, Gaworska AVA, Roberts NW, Whitney HM, Partridge JC (2014) Bumblebees Learn Polarization Patterns. *Curr Biol* 24: 1415–1420
- Gemeno C, Yeargan KV, Haynes KF (2000) Aggressive chemical mimicry by the bolas spider *Mastophora hutchinsoni*: identification and quantification of a major prey's sex pheromone components in the spider's volatile emissions. *J Chem Ecol* 26: 1235–1243
- Gullan PJ, Cranston PS (2010) *The Insects: An Outline of Entomology*. John Wiley & Sons, New Jersey
- Haynes KF, Gemeno C, Yeargan KV, Millar JG, Johnson KM (2002) Aggressive chemical mimicry of moth pheromones by a bolas spider: how does this specialist predator attract more than one species of prey? *Chemoecology* 12: 99–105
- Heiling AM, Herberstein ME, Chittka L (2003) Crab-spiders manipulate flower signals. *Nature* 421: 334
- Heiling AM, Chittka L, Cheng K, Herberstein ME (2005) Colouration in crab spiders: substrate choice and prey attraction. *J Exp Biol* 208: 1785–1792
- Honkavaara J, Koivula M, Korpimäki E, Siitari H, Viitala J (2002) Ultraviolet vision and foraging in terrestrial vertebrates. *OIKOS* 98: 505–511
- Hurd LE, Prete FR, Jones TH, Singh TB, Co JE, Portman RT (2004) First identification of a putative sex pheromone in a praying mantid. *J Chem Ecol* 30: 155–166
- Keeling CI, Otis GW, Hadisoelilo S, Slessor KN (2001) Mandibular gland component analysis in the head extracts of *Apis cerana* and *Apis nigrocincta*. *Apidologie* 32: 243–252
- Kevan PG, Baker HG (1983) Insect as flower visitors and pollinators. *Ann Rev Entomol* 28: 407–453
- Kinoshita M, Takahashi Y, Arikawa K (2011) Simultaneous brightness contrast of foraging *Papilio* butterflies. *P R Soc B* 279: 1911–1918
- Lunau K (1996) Signalling functions of floral colour patterns for insect flower visitors. *Zool Anz* 235: 11–30
- Maxwell MR, Barry KL, Johns PM (2010) Examinations of female pheromone use in two praying mantids, *Stagmomantis limbata* and *Tenodera aridifolia sinensis* (Mantodea: Mantidae). *Ann Entomol Soc Am* 103: 120–127
- Milet-Pinheiro P, Ayasse M, Schindwein C, Dobson HEM, Dötterl S (2012) Host location by visual and olfactory floral cues in an oligolectic bee: innate and learned behavior. *Behav Ecol* 23: 531–538
- O'Hanlon JC, Li D, Norma-Rashid Y (2013) Coloration and morphology of the Orchid Mantis *Hymenopus coronatus* (Mantodea: Hymenopodidae). *JOR* 22(1): 35–44
- O'Hanlon JC, Holwell GI, Herberstein E (2014a) Pollinator deception in the Orchid Mantis. *Am Nat* 183: 126–132
- O'Hanlon JC, Holwell GI, Herberstein ME (2014b) Predatory pollinator deception: does the orchid mantis resemble a model species? *Acta Zool Sinica* 60: 90–103
- Owen D (1980) *Camouflage and Mimicry*. The University of Chicago Press
- Perez B (2005) Calling behaviour in the female praying mantis, *Hierodula patellifera*. *Physiol Entomol* 30: 42–47
- Rae JM, Vamosi JC (2013) Ultraviolet reflectance mediates pollinator visitation in *Mimulus guttatus*. *Plant Spec Biol* 28: 177–184
- Rakosy D, Streinzer M, Paulus HF, Spaethe J (2012) Floral visual signal increases reproductive success in a sexually deceptive orchid. *Arthropod-Plant Int* 6: 671–681
- Sasaki M, Ono M, Asada S, Yoshida T (1991) Oriental orchid (*Cymbidium pumilum*) attracts drones of the Japanese honeybee (*Apis cerana japonica*) as pollinators. *Experientia* 47: 1229–1231
- Spaethe J, Tautz J, Chittka L (2001) Visual constraints in foraging bumblebees: Flower size and color affect search time and flight behavior. *P Natl Acad Sci USA* 98: 3898–3903
- Spaethe J, Moser WH, Paulus HF (2007) Increase of pollinator attraction by means of a visual signal in the sexually deceptive orchid, *Ophrys heldreichii* (Orchidaceae). *Pl Syst Evol* 246: 31–40
- Spaethe J, Streinzer M, Paulus HF (2010) Why sexually deceptive orchids have colored flowers. *CIB* 3: 139–141
- Stevens M, Merilaita S (2009) Animal camouflage: current issues and new perspectives. *Phil Trans R Soc B* 364: 423–427
- Stoddard MC (2012) Mimicry and masquerade from the avian visual perspective. *Curr Zool* 58: 630–648
- Stowe MK, Tumlinson JH, Heath RR (1987) Chemical mimicry: bolas spiders emit components of moth prey species sex pheromones. *Science* 236: 964–967
- Streinzer M, Paulus HF, Spaethe J (2009) Floral colour signal increases short-range detectability of a sexually deceptive orchid to its bee pollinator. *J Exp Biol* 212: 1365–1370
- Sugahara M, Park IH, Chen Y, Kongpitak P (2006) Aggregation activity of *Apis cerana* subspecies on *Cymbidium floribundum*. *Honeybee Sci* 26: 159–162
- Sugahara M, Izutsu K, Nishimura Y, Sakamoto F (2013) Oriental Orchid (*Cymbidium floribundum*) attracts the Japanese Honeybee (*Apis cerana japonica*) with a mixture of 3-hydroxyoctanoic acid and 10-hydroxy-(E)-2-decenoic acid. *Zool Sci* 30: 99–104
- Watanabe H, Yano E (2013) Behavioral response of mantid *Tenodera aridifolia* (Mantodea: Mantidae) to windy conditions as a cryptic approach strategy for approaching prey. *Entomol Sci* 16: 40–46
- Wickler W (1968) *Mimicry in Plants and Animals*. World University Library, London
- Yamamura K (1999) Transformation using (x 0.5) to stabilize the variance of populations. *Res Popul Ecol* 41: 229–234

(Received June 10, 2014 / Accepted August 14, 2014)