

Experimental demonstration of close-range olfaction and contact chemoreception in the Brazilian harvestman, *Iporangaia pustulosa*

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Abstract

We studied the ability to detect food by close-range olfaction and contact chemoreception in the harvestman *Iporangaia pustulosa* Mello-Leitão (Opiliones: Laniatores: Gonyleptidae). We first tested the reaction of individuals towards tasteless (pure agar), aversive (agar with salt), and food-intake stimulating substrates (agar with saccharose). Only the substrate containing saccharose was consumed. Contact (mainly with legs II) was necessary for detection of the agar and, before ingestion, the stimulus was always tapped with legs I. In the second experiment, we observed the behavior of individuals in an arena with a screened plastic box containing pieces of *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae. Individuals spent more time on the box containing food than on the control. In the third experiment, in an arena identical to that used in Experiment 2, we introduced a live but motionless *T. molitor* larva in the box. There was no difference between experimental and control treatments. We also observed the behavior of *I. pustulosa* in an arena containing live isopods. In first capture attempts, isopods were only detected upon contact, mainly with legs I. Our results suggest that (i) *I. pustulosa* is capable of detecting food only by its chemical properties; (ii) food with weak odor may not be detected by close-range olfaction; and (iii) legs I and II are important for food detection but, before ingestion, legs I are used to examine potential food items.

Introduction

Chemical cues are widely used in arthropods for the detection of food (Steidle & van Loon, 2003), and are important because they may provide more specific information than mechanical cues (e.g., Eisner et al., 2004; Willemart & Pellegatti-Franco, 2006:32). In arachnids, most studies on chemoreception have focused on scorpions, mites, and spiders (e.g., Krapf, 1986; Dicke et al., 2000; Persons & Rypstra, 2000), although harvestmen are especially interesting in studies of the evolution of feeding strategies in arachnids. They are the only omnivorous clade of the 11 suborders of Arachnida. Thus, their adaptations to omnivory probably originated from a predatory ancestor (Willemart et al., in press). We expect that predatory and generalist feeders should bear distinct sensory structures and capabilities, foraging strategies, and strike ability in order to capture mobile prey. In this

scenario, studying chemoreception of harvestmen is a first step towards understanding the ultimate and proximate causation (sensu Wilson, 1975) of omnivory within the group.

The order Opiliones is divided into four suborders, namely, Cyphophthalmi, Eupnoi, Dyspnoi, and Laniatores (Giribet et al., 2002). They are mainly nocturnal and non-visual animals that inhabit humid environments (Berland, 1949; Hillyard & Sankey, 1989). Although some species are food specialists (e.g., Nyffeler & Symondson, 2001), most species feed opportunistically on animal and plant matter (see references in Gnaspi, 1996; Newton & Yeagan, 2001), and it has been reported that harvestmen are highly dependent on contact stimuli to receive information from the environment (Phillipson, 1960; Macías-Ordóñez, 1997, 2000). Mechanical stimuli in species of all four suborders of harvestmen can be perceived by sensilla chaetica, which are capable of deflecting in all directions after contact (Barth & Blickhan, 1984; Eisenbeis & Wichard, 1987; Guffey et al., 2000; Willemart & Gnaspi, 2003; de Bivort & Giribet, 2004). Sensilla chaetica may also function as

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contact chemoreceptors, as evidenced by the presence of particular morphological and histological features (Spicer, 1987; Guffey et al., 2000; Willemart & Gnaspini, 2003). Pabst (1953) reported harvestmen following snail trails, and Willemart (2002) observed individuals of *Heteromites discolor* (Sørensen) using their chelicerae to scrape stones where pieces of food had previously been deposited, and also to detect chemical trails left by food dragged by another harvestman. Moreover, Immel (1953, 1954) demonstrated that *Paranemastoma quadripunctatum* Perty rejects agar without food extracts while accepting agar with food extracts.

Concerning olfaction, Foelix (1976) has reported olfactory setae in Cyphophthalmi, Spicer (1987) and Holmberg & Cokendolpher (1997) have reported possible olfactory receptors in the pedipalps of some Eupnoi, and Immel (1953) has demonstrated avoidance of xylol prior to contact in a Dyspnoi. No olfactory receptors have been described in the suborder Laniatores. Contact chemoreceptors may also respond to high concentrations of some odors (Chapman, 1998), and Willemart (2002) has observed, in closed terraria, that harvestmen were attracted from a distance to motionless food, such as dead prey or artificial food. We therefore predict that food surrounded by a high-odor concentration can be detected by harvestmen.

Based on the morphological and behavioral evidence cited, we aimed at testing two hypotheses with *Iporangaia pustulosa* Mello-Leitão (Opiliones: Laniatores: Gonyleptidae) as experimental organism (i) that a laniatorid harvestman is able to detect food by contact chemoreception and (ii) that a laniatorid harvestman is able to detect food by close-range olfaction.

Materials and methods

Collection and maintenance of the harvestmen and general methods

Iporangaia pustulosa were used as a model because of their general occurrence in specific known sites. The individuals were collected in April 2004 and January 2005, in the Parque Estadual Intervales, Ribeirão Grande, state of São Paulo, Brazil, along the banks of a river on the upper or inner part of leaves in shrubs or trees during the day. They were maintained in terraria with rocks and soil on the bottom, and sticks and styrofoam bars crossing the terraria. Males and females were kept in separate terraria. They were fed ad libitum on dead isopods, cooked potatoes, and rice, and the relative humidity of the terraria was maintained at 80–95% by keeping the soil humid. The animals tested were starved for 14–15 days before each experiment. Direct observations on behavior towards isopods were conducted 1–2 days following the experiments, thus after 16–17 days of starvation. We used a long

starvation period to minimize the possibility that a test animal would ignore the food because it was not hungry instead of not having detected it (see Saxena, 1967).

All experiments and direct observations were conducted at room temperature (20–25 °C). As *I. pustulosa* is known to display both nocturnal and diurnal activity (Hoenen & Gnaspini, 1999; Machado et al., 2004), we conducted the experiments according to the observed activity in captivity in the days before the experiments/observations. Each group of individuals was only used in one of the four experiments/observations (1, 2A, 2B and ‘Direct observations’).

Experiment 1: contact chemoreception

In this experiment, we wished to verify if the animal tested would show distinct behaviors towards blocks of agar containing a feeding stimulant (saccharose), a substrate containing only water and agar, and a substrate containing a feeding inhibitor (salt). To obtain a solid block of agar, we used 1 g of agar per 100 ml of distilled water. For the stimulatory substrate, we added 1 g of saccharose per 60 ml of the solution described above. For the inhibitory stimulus, we added 1 g of salt per 20 ml of the agar solution. We used a low concentration of saccharose to avoid a high-vapor concentration above the piece of agar and a high concentration of salt to make it clearly different from the substrate containing only agar. The substrates were prepared within 12 h before the experiments and kept in the refrigerator until the beginning of the tests. The test arena was an uncovered terrarium of 20 × 14 × 14 cm with humid paper towelling on the bottom. Each individual harvestman was introduced in a glass vial in the center of the terrarium and left to acclimatize for 3 min, after which it was released and allowed to wander in the terrarium until it touched a block of agar. Two identical agar blocks of 10 × 10 × 2 mm were placed in opposite corners of the terrarium. We started video recording every time the animal approached the agar. We quantified which leg first contacted the agar, which leg touched the agar after the first contact, and whether or not the test animal ingested the agar. We considered that a ‘bout-of-leg-I tapping’ occurred when the animal repeatedly (more than twice with each leg) touched the agar with dorsal, tip, or ventral regions of leg I. We defined ‘ingesting’ as remaining at least 3 min with the mouthparts in contact with the agar. Twenty-four individuals were tested per day, 12 of each sex, in a way that each group of four individuals of each sex received treatments in the following orders: agar only – saccharose – salt; salt – agar only – saccharose; and saccharose – salt – agar only, with an interval of 20–23 days between each testing day. We conducted the experiments during the day (between 08:00 and 16:00 hours). After each test, the paper towel was changed, and both the

terrarium and the glass vial were cleaned with 90% ethanol and were allowed to dry before the following experiment.

Experiment 2: olfaction

A-Strong odor. In this experiment, we wanted to verify if the test animal would show interest in a small screened box containing food. We used an uncovered terrarium of $20 \times 14 \times 14$ cm with humid paper towelling on the bottom. In the center of the terrarium, we put a plastic box (LEGO®, Billund, Denmark) of $3.2 \times 3.2 \times 2$ cm in height, closed on top by a screen (0.41 mm aperture). In the experimental treatment, we proceeded as follows: 24 h before the test, we cut a *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larva (weight: 0.20 g, fed on bread) into six pieces and left it in a closed plastic container at room temperature (ca. 20 °C) and 100% r.h. Each larva was only used in three consecutive tests. In the control treatment, we left a humid sheet of paper towel of 0.5×0.5 cm in the box (humidity control), replacing it by a new one after every three tests. Around this box, we delimited a square arena on the ground of 64 cm^2 to check the time the animals would spend close to the box. An individual was introduced in a glass vial in one of the corners of the terrarium (chosen at random) and left in it for acclimatization for 3 min, after which it was released and video recorded for 15 min. The terrarium, glass vial, box, and the arena were cleaned with ethanol 90% and were allowed to dry before the following experiment. Fifteen individuals were tested in both control and experimental treatments. We conducted the experiments at night (between 21:00 and 04:00 hours) under red light. Half the individuals chosen at random were first tested under experimental conditions and during the following night under control conditions. The other half was first tested under control conditions, and in the following night under experimental conditions (see Martin & Bateson, 1996).

B-Weak odor. The experiment was conducted as above except that the stimulus used was a live *T. molitor* larva about to pupate (weight: 0.19 g; fed on bread). In contrast with the dead *T. molitor* larvae cut in pieces and left under high humidity for 24 h before the experiment, we assumed this stimulus to have a weak odor. Larvae about to molt do not move at all unless touched (RH Willemart, unpubl.). The same larva was used for all the harvestmen tested. The larva did not move throughout the experiments, even during cleaning of the exterior part of the box with ethanol. It molted a few days after the experiments. The use of a motionless larva was important to minimize mechanical stimuli such as substrate-borne vibrations or air movements.

Direct observations with live prey

Here we wished to verify if *I. pustulosa* was able to detect live prey without touching them. Live prey being presumably a food source releasing little odor, we inferred that detection without contact would probably be by mechanical clues such as substrate-borne vibrations and/or air displacement. We introduced an individual of *I. pustulosa* in a glass vial into a terrarium of $20 \times 14 \times 14$ cm with humid soil on the bottom, and allowed it to acclimatize for 3 min. Before releasing the test animal, we introduced two isopods into the terrarium – previous observations had shown that these were not detected by harvestmen from a distance (>5 cm from the tip of a leg). Therefore, there would be no interference of stimuli between them, such as, for example, substrate-borne vibrations coming from isopods on opposite sides of the terrarium. When an individual *I. pustulosa* approached an isopod, we started video recording. We quantified if the harvestman detected the isopods without contact, which leg first contacted the isopod, which leg touched the isopod after the first contact, and the number of attempts and successful captures by the harvestmen. Detection was inferred by a characteristic behavior of the harvestmen: directing legs I and/or II towards the isopod and extending the pedipalps. All relevant behaviors that followed capture attempts were also recorded. Ten females and 15 males were observed. The individuals remained no more than 10 min in the arena. We conducted these observations during the day (between 08:00 and 16:00 hours). Both the terrarium and the glass vial were cleaned with 90% ethanol and were allowed to dry before the following observation. Soil was changed between observations, and isopods that were touched by the harvestmen were not used again.

Measurements of legs and data analysis

Because the distinct use of each leg pair could be related to its length, we measured the length of all four legs on the right side of 15 males and 10 females with a digimatic caliper. We used SigmaStat statistical software, version 2.0 (Systat Software Inc., Point Richmond, CA, USA), with α set at 0.05. We used medians instead of averages to present our results, as data were not normally distributed (Zar, 1996). Data from males and females of the experiments were pooled as there was no significant difference between them in any comparison ($P > 0.05$).

Results

Experiment 1: contact chemoreception

The individuals never detected (see definition of ‘detection’ in the Materials and methods) the agar unless they touched it ($n = 24$ for each of three treatments, 100%

of the cases). Legs II were the first to contact the agar in 44 cases (62%), legs I in 25 (35%), and legs III in two cases (3%). All individuals fed on the agar that was supplemented with saccharose in all cases after displaying a bout-of-leg-I tapping ($n = 24$). No individual fed on the agar-only substrate, and 12 out of 24 displayed a bout-of-leg-I tapping on it. No individual fed on the agar containing salt either, and 14 out of 23 displayed leg-I tapping on it. The median of the time spent displaying a bout-of-leg-I tapping was 7.3 s for the saccharose substrate (range 2.1–25.7 s; $n = 23$), 12.7 s for the agar-only substrate (range 2.1–55.2 s; $n = 12$), and 14.2 s for the salt substrate (range 5.3–36.0 s; $n = 14$) (Kruskal–Wallis: $H = 10.37$, d.f. = 2, $P = 0.006$; data assumed to be independent as there was an interval of 20–23 days between subsequent treatments). In 10 out of 50 cases in which a bout-of-leg-I tapping occurred, the individual also touched the agar with leg II, but with no more than three brief taps (no difference between treatments: $\chi^2 = 0.991$, d.f. = 2, $P = 0.609$).

Experiment 2: olfaction

Strong odor. The individuals spent an equal amount of time in the square area around the box (Wilcoxon two-sample test: $W = 30.0$, $P = 0.421$; $n = 15$). However, they did spend significantly more time on the box containing food than on the control box ($W = -89.0$, $P < 0.001$; $n = 15$).

Weak odor. Again, the individuals spent an equal amount of time in the square ($W = 3.0$, $P = 0.922$; $n = 15$). This was also true for the time spent on the box in the experimental treatment and the control treatment ($W = 10.0$, $P = 0.125$; $n = 15$).

Direct observations

On first capture attempts, isopods were never detected unless touched ($n = 15$; in five cases the isopods were not touched and therefore not detected; in five cases we do not know whether the harvestmen detected the isopod before touching it). In 12 cases, legs I were the first to make contact with the isopod, and three times legs II made the first contact. In 13 cases, harvestmen legs I and II were less than 1 cm from the isopods (moving or motionless) while no detection was observed (data pooled from 11 individuals). In 11 out of 13 cases that we could record, the first pair of legs was used to touch the isopod following the first contact, and in two cases both legs II (only one tap) and I were used. First capture attempts (with the harvestmen extending the pedipalps and stroking the prey) were never successful, the isopods either fled by running ($n = 11$) or were grabbed by the pedipalps but managed to escape

($n = 4$). When the harvestmen managed to pursue the isopod by maintaining contact with legs I and II, they attempted to capture it again. From the second trial on, the individuals captured the isopods in 2 out of 15 attempts (data pooled from eight individuals). On second attempts, harvestmen were able to detect isopods by waving legs I above them (< 1 cm) without contact ($n = 9$; data pooled from seven individuals). When the isopods fled and the harvestmen were no longer able to touch or wave the legs above them, they did not find them again ($n = 9$).

Leg measurements

The following leg lengths were found: legs I: 17.4 ± 0.7 mm (range 16.2–18.5); legs II: 44.4 ± 1.9 mm (range 49.1–41.4); legs III: 28.9 ± 1.1 mm (range 27.2–31.6); legs IV: 40.2 ± 1.5 mm (range 43.3–37.8). Legs differed significantly in their length (Kruskal–Wallis: $H = 91.95$; $P < 0.001$; $n = 25$), whatever leg pair was compared (Dunn's test: $P < 0.05$).

Discussion

Importance of legs I and II

Laniatorid harvestmen are known to have short legs I and elongated legs II, relative to legs III and IV (e.g., Gnaspini, 1995), which is supported by our data. The latter two are used to support the body, whereas the first two are mainly sensory (see discussion in Willemart & Gnaspini, 2003). It has been proposed by Guffey (1999) that legs II would be responsible for perception of the general features of the environment, whereas legs I would be responsible for fine analysis of resources. This is in accordance with the length of these legs, as longer legs are more appropriate for the first purpose and shorter legs fit better the role of analyzing a food item or other resources that is a few millimeter, in front of the body. Despite the traditional view that legs II are the sensory legs of harvestmen (e.g., Cloudsley-Thompson, 1958; Kästner, 1968; Goodnight & Goodnight, 1976; Elpino-Campos et al., 2001) it is clear from our data that legs I are also important for detailed evaluation/recognition of the food, as these always touched the food before the animal started feeding. Both legs I and II seem to be important for food detection, because legs I first contacted the isopods in 62% of the cases (in the Direct observations), and legs II first contacted the agar in 80% of the cases (in Experiment 1).

Contact chemoreception and food recognition

Though there might be mechanical and olfactory cues available, the final decision concerning accepting/rejecting a food item in arthropods usually occurs after contact chemoreception or gustation (Anton & Gnatzy, 1998; van

Loon & Dicke, 2001). Therefore, we expect the ability of perceiving the chemical properties of food upon contact to occur in harvestmen (Foelix, 1985). In our experiments, because the harvestmen did not detect the agar before touching it, we conclude that the agar was not producing detectable odors. Behaviors towards the salt and pure agar substrates were similar, namely quickly rejecting it or analyzing it carefully and thereafter rejecting it (median of 12.7–14.2 s for a bout-of-leg-I tapping). These similar behaviors could be explained by the fact that both agars were recognized as unprofitable resources. In contrast, saccharose was readily recognized after only approximately 7 s of leg-I tapping, after which it was always consumed. Contact chemoreception is particularly important in harvestmen, as they may feed on feces, remains of dead animals, and fruits (see references in Willemart, 2002), items that do not have specific size, format, or texture and thus would not be recognizable by their physical properties. Moreover, contact is often necessary to elicit the attack response (e.g., Acari: Usher & Bowring, 1984; Baatrup et al., 2006; Opiliones: Phillipson, 1960), and thus appropriate recognition by chemoreception also aids in preying on live animals.

Olfaction and foraging ability

Our results suggest that, during first capture attempts, food sources emitting a weak odor might not be detected by the harvestmen unless contact is made and that only strongly scented food is detected without contact. Therefore, it may be that live invertebrates are hardly detected by olfaction, whereas dead prey items, strong-scented fruits, and feces produce sufficiently high-odor concentrations to allow olfaction-based detection. The fact that harvestmen were able to detect isopods at <1 cm distance in capture attempts that followed first contact with prey could be due to (i) a shift in sensitivity and/or (ii) the release of secretions by the isopods following first capture attempts by the harvestmen (see, e.g., Wisenden et al., 1999).

One could expect a high mobility in animals in which motionless food is an important part of the diet, to enhance the probability of encounter (see Riechert & Luczak, 1982 for discussion). Moreover, in generalist feeders random search might be a better strategy than focusing on specific cues (Steidle & van Loon, 2003), as generalists do not search for stimuli of a specific prey or plant used by this prey (e.g., McGregor & Gillespie, 2004). While foraging, harvestmen alternate between motionless and wandering phases (see Willemart et al., in press) and probe the environment constantly with legs II and/or I and/or pedipalps (Hillyard & Sankey, 1989; Guffey, 1999; Willemart, 2002). These behaviors probably increase the chances of

finding motionless food, which is readily detected upon contact or at close range through odor production.

Scorpions and spiders are known to detect prey at a distance, are fast enough to capture them, and feed mostly on wandering prey (e.g., Brownell, 2001; Barth, 2002). *Iporangaia pustulosa* did not detect live prey prior to contact, and following detection by contact, they were not fast or powerful enough to capture this prey. Also, as mentioned above, motionless food seem to be an important part of the laniatorid diet. Therefore, reduced sensory capabilities to detect physical and chemical cues at a distance (harvestmen are non-visual animals) and reduced rapidity possibly played an important role in the evolution of feeding behavior and diet breadth of these harvestmen.

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