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SHORT COMMUNICATION

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Alarm communication: a new function for the scent-gland secretion in harvestmen (Arachnida: Opiliones)

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Abstract Most harvestmen are nocturnal, nonacoustical, and nonvisual arthropods. They have a pair of exocrine glands on the cephalothorax that produce defensive volatile secretions. We investigated in the field the possible alarm effect of these secretions in the gregarious harvestman *Goniosoma* aff. *proximum*. A cotton swab soaked with the species' own exudate (treatment), or with water (control), was held 1–2 cm from the center of harvestmen aggregations. The results showed that the gland secretion elicits an alarm response in *Goniosoma*: whereas 73.3% of the aggregations dispersed after being stimulated with the gland exudate, only 3.3% responded to the water control. Respondent groups are larger than nonrespondent groups, and the time of reaction to the secretion was inversely related to group size. This is the first demonstration of a chemically-mediated alarm effect in harvestmen. The alarm response in gregarious harvestmen has possibly evolved as a by-product of a primarily defensive reaction in the context of predator avoidance. The discovery of this novel function of scent-gland secretion is meaningful in view of the widespread occurrence of gregarious habit among species of the order Opiliones.

Introduction

Over half of all terrestrial arthropod orders contain species that use chemical deterrents for defense. Most of these products are secreted by exocrine glands and are often complex mixtures that serve multiple roles (Blum 1981). In fact, for many arthropods, compounds that were once believed to be primarily defensive have been subsequently found to function parsimoniously as chemical signals associated with intraspecific communication (Whitman et al. 1994). Such signals are known as pheromones (sensu Karlson and Lüscher 1959), and may be used in a variety of contexts, including sexual, gregarious, alarm, or territorial behavior (Blum 1985).

The species of the order Opiliones are characterized by a pair of exocrine glands located at the anterior margins of the cephalothorax near the base of the second pair of legs (Shultz 1990). These glands produce a variety of volatile secretions that are released under the threat of predation (Eisner et al. 1971, 1978; Acosta et al. 1993; Machado et al. 2000). Most harvestmen are nocturnally active, nonacoustical and nonvisual, and have a long slender second pair of antenniform legs. The combination of these features suggests that olfaction may be an important sensory modality for intraspecific communication in these animals.

Bishop (1950) was the first to suggest that the scent-gland secretions in harvestmen could be used for intraspecific communication and proposed that individuals could deposit chemical signals on the ground as trail markers. It has also been suggested that such secretions could be used for sexual recognition or as an attraction pheromone (Holmberg 1986). However this hypothesis is unlikely, since other glandular structures (on the chelicerae and legs) have been found that serve these functions (Martens and Schawaller 1977; Martens 1979). Moreover, there is no chemical difference between the secretions from males and females (Meinwald et al. 1971), as would be expected if the substances had a sexual role (Blum 1985).

Many harvestmen species show gregarious habits and form dense diurnal aggregations consisting of nymphs and adults of both sexes (Cockerill 1988; Machado et al. 2000 and references therein). There is evidence suggesting that gregariousness may be related to the choice of suitable microconditions or to group defense (Holmberg et al. 1984; Machado et al. 2000). Wagner (1954) postulated that harvestmen are attracted to aggregation sites by the scent of odoriferous glands. However, it is also possible that the secretion could be used in the opposite way, eliciting an alarm response upon disturbance of the

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group. Indeed, group living is a prerequisite for the evolution of alarm signals, and these substances have been identified in many gregarious species of treehoppers, aphids, true bugs, water-striders, and social insects, such as termites, wasps, bees, and ants (Blum 1969; Hölldobler 1977). In these groups, chemical alarm signals are generally perceived by multiporous sensilla located on the antennae and may result in attack or dispersion (Blum 1985). Moreover, the propensity to respond to the alarm signal increases with group size (Vulinec 1990). Among the major arachnid groups, chemical alarm signals are only known in the Acari (Noguchi et al. 1998).

Species of harvestmen in the Neotropical genus *Goniosoma* are highly gregarious, and normally take shelter inside caves, rock crevices, and tree trunks (Pinto-da-Rocha 1993; Gnaspini 1996; Machado and Oliveira 1998; Machado et al. 2000). Chemical studies have shown that the secretions produced by *Goniosoma* are mainly quinones (Gnaspini and Cavalheiro 1998), which are widespread predator deterrents among arthropods (Blum 1981). In this study we address three questions: (1) Does the defensive secretion produced by *G.* aff. *proximum* elicit an alarm response in aggregated individuals? (2) Are respondent groups larger than non respondent ones? (3) Is there a direct relation between aggregation size and time of response?

Materials and methods

Fieldwork was carried out in the rainforest of Cardoso Island (25°18′ S; 48°05′ W), on the south coast of São Paulo state, SE Brazil, from January to July 2000. Field observations and experiments were conducted during daylight in a 2 km transect along a 6 m wide stream. Voucher specimens are deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP), Brazil.

We considered as an aggregation any group of at least three harvestmen whose legs were overlapping (Machado et al. 2000). The role of the defensive secretion in alarm communication was evaluated through a field experiment in which aggregations of *G.* aff. *proximum* were exposed to the species' own gland exudate (treatment), or to distilled water (control). We used water as control because many harvestmen species dilute their own gland secretion in aqueous enteric fluid (Eisner et al. 1971, 1978; Gnaspini and Cavalheiro 1998). In *G.* aff. *proximum*, the chemical composition of the repugnant secretion consists of two pairs of isomers of alkyl-1,4-benzoquinones (MW 136 and 150; G. Machado and J.R. Trigo, unpublished data). Most likely the isomers of mass 136 are 2,3- and 2,5-dimethyl-benzoquinone, as reported for other harvestmen (see Eisner et al. 1978), and hence the two isomers of mass 150 are probably the corresponding quinones with one methyl group replaced by an ethyl group.

Aggregations were randomly designated by the flip of a coin as treatment or control. Aggregation size did not differ significantly between treatment and control groups ($\bar{X}$±SD=17.9±11.3 vs. 23.1±23.9 individuals, respectively; *t*-test: *t*=1.08, *df*=58, *P*=0.28). Immediately before each trial, two previously collected individuals of *G.* aff. *proximum* were milked of secretion by seizing them by hand. The fluid that oozed from the harvestmen's glands was then deposited on a cotton swab (20 cm long). The tip of the cotton swab, the point of emission of the evaporating secretion, was held 1–2 cm from the center of an aggregation of harvestmen resting on the rock surface (*n*=30 aggregations; Fig. 1). The same procedure was repeated using a cotton swab dampened with distilled water as control (*n*=30 aggregations). A new cotton swab was used in each trial of either experimental group, and no harvestmen aggregation was tested more than once. Since repeated milking of the same 'source individuals' could reduce the concentration of the chemical compound secreted, two new unmilked harvestmen were used for each trial.

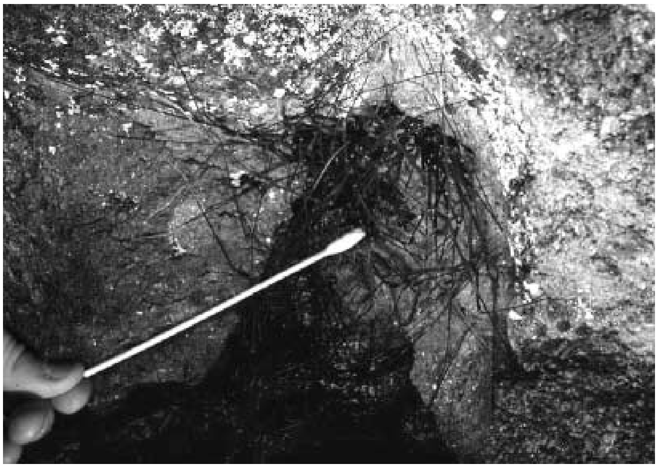

Fig. 1 Experimental method for testing the alarm effect of the scent-gland secretion of the harvestman *Goniosoma* aff. *proximum*. Aggregated individuals in the treatment group (*n*=30 aggregations) were stimulated with a cotton swab (20 cm long) soaked with the species' own secretion, while individuals in the control group (*n*=30 aggregations) were exposed to a water-soaked cotton swab

The behavior of the individuals was recorded during a 60 s period from the presentation of the swab, after which the trial terminated. Aggregations were categorized in two behavioral groups: (1) individuals in the 'respondent' group abandoned the resting location, and ran away at least 50 cm from the periphery of the aggregation; (2) individuals in the 'non-respondent' group remained motionless during and after the trial. The number of individuals of all tested aggregations was counted both before and after each trial. Since it is not easy to distinguish or define alarm in a bioassay (Billen and Morgan 1998), we adopted the concept of 'panic alarm' proposed by Wilson and Regnier (1971) in which respondent animals present excited bursts of nondirectional running.

Results

The mean number of *Goniosoma* individuals per aggregation was 19.45±18.40 (range 3–79 individuals; *n*=82 aggregations) and the mean sex ratio was nearly 1:1 ($\bar{X}$±SD=1.07±1.39, range 0.25–7, *n*=51). Harvestmen usually aggregated close to the water (range 0.02–2.0 m, *n*=82 aggregations) on exposed rocks or, more frequently, inside cracks. When disturbed by intensive exposure to light or touching, aggregated individuals ran away or occasionally fell from the rock into the river. Individuals also collectively discharged scent-gland secretions upon manipulation.

The results of the experiment revealed that the scent-gland secretion unequivocally elicits an alarm response in *G.* aff. *proximum*. In 22 out of 30 aggregations (73.3%), the individuals in the center of the group dispersed rapidly after being stimulated with the gland exu-

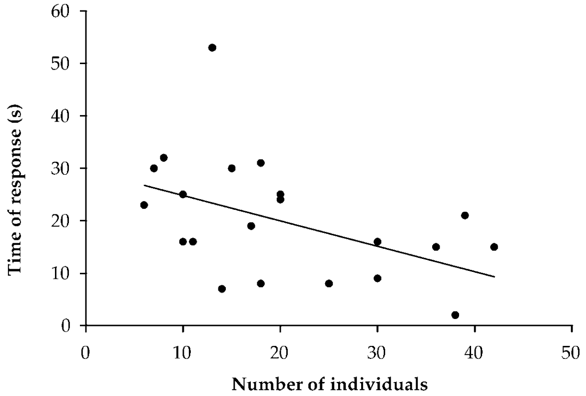

Fig. 2 Relationship between group size and time of response by aggregated individuals of the harvestman *Goniosoma* aff. *proximum*, upon stimulation with a cotton swab impregnated with the species' own scent-gland secretion

date. On the other hand, only one of 30 aggregations (3.3%) responded to the water-soaked cotton swab (Yates corrected χ^2=31.09, *df*=1, *P*<0.0001). Upon chemical stimulation the respondent individuals quickly ran away from their resting location (*X*±SD=19.68±11.49 s, range 2–53 s, *n*=22). Given that the alarmed harvestmen bump into other individuals, the alarm reaction is also probably mechanically spread through the aggregation, resulting in a general erratic scattering of the group.

Our experiments with *Goniosoma* also showed that respondent aggregations (*X*±SD=20.55±11.10, range 6–42 individuals, *n*=22) had significantly more individuals than non-respondent ones (*X*±SD=10.50±8.55, range 4–30 individuals, *n*=8) (Mann-Whitney test: *U*=142.0, *P*<0.02). Moreover, among respondent groups, the time to react to the scent-gland secretion was inversely related to group size (Pearson correlation: *r*=–0.467, *n*=22, *P*<0.03) (Fig. 2).

Discussion

Harvestmen scent-gland secretions can provide an effective defense against ants and other invertebrate and vertebrate predators (Eisner et al. 1978; Holmberg 1986). Several studies have shown that scent-gland secretions by harvestmen are only discharged after physical contact with a potential predator (Eisner et al. 1971, 1978; Duffield et al. 1981). In this study, we demonstrated that the defensive secretion can also elicit alarm behavior among aggregated individuals, causing them to disperse from a chemically marked, and presumably risky, area. Signals that are released upon mechanical damage to the sender are known as damage-release alarm signals, and act as reliable indicators of predation risk, since they indicate that a nearby conspecific was recently attacked (Chivers et al. 1996). This is the first demonstration of such a chemically-mediated signal in harvestmen. The discovery of this novel function of scent-gland secretion is meaningful in view of the widespread occurrence of gregarious habit among species in the order Opiliones (Holmberg et al. 1984; Cockerill 1988; Machado et al. 2000).

We also demonstrated for the first time among arachnids that the reaction response to the alarm signal varies with the size of the group. Larger groups apparently react faster to the chemical signal as a consequence of the increased number of sensorial legs used for surveillance. An analogous behavior is observed in other arthropods such as water-striders, in which large groups respond faster than small groups to an approaching predator, due to an increased visual surveillance (Vulinec 1990). Such a positive relationship between number of individuals and promptness of reaction is also well documented among visually-oriented vertebrates (Kenward 1978; Hoogland 1981).

Gregariousness in harvestmen may also produce a dilution effect by reducing the probability of attack upon an individual as group size increases, and enhances escape capability of aggregated individuals due to alarm communication after predator attack. The mechanical propagation of the alarm response among the aggregated harvestmen is similar to the so-called 'Trafalgar effect' (sensu Treherne and Foster 1981). Such an effect also occurs in tight aggregations of thysanurans, aphids, and water-striders in which the disturbance promoted by body contacts is used as a cue for approaching danger (reviewed by Vulinec 1990).

Apart from the deterrent function of such secretions, one might still ponder about an individual harvestman's advantage in chemically signaling to neighboring conspecifics upon a predator attack. Is there any additional benefit for the sender? As stressed by Blum (1985) for many insects, our results suggest that scent-gland secretion in *Goniosoma* still functions as a defensive allomone, its pheromonal role probably being secondarily derived. The alarm effect in harvestmen has probably evolved as a by-product of a primarily defensive reaction in the context of predator avoidance. If there is an individual advantage in signaling, this would probably depend on a confusion effect on the predator generated by the general fleeing, and/or on a possible benefit to the sender due to the escape of genetically related individuals in the aggregation.

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