Defences of a Neotropical harvestman against different levels of threat by the recluse spider

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Abstract

The threat sensitive hypothesis predicts that animals modulate the defensive behaviour with the level of threat. Therefore, responses to predator cues may differ from responses to the actual predator in close range. Also, in high threat situations, prey would be expected to use their most dangerous defences. The recluse spider \textit{Loxosceles gauch\textsuperscript{o}} (Araneae, Sicariidae) is known to prey upon well defended harvestmen such as the laniatorid \textit{Mischonyx cuspidatus} (Opiliones, Gonyleptidae), which has been reported to use tanathosis, chemical defences, pinching with sharp apophyses on legs, chelicerae and pedipalps. Because of harvestmen’s dependence on chemical stimuli, we tested if \textit{M. cuspidatus} would change its locomotory behaviour in the presence of chemicals of the recluse spider (low threat situation: spider vs blank vs chemical control; one at a time). Subsequently, we tested harvestmen behaviour in the presence of the spider in close range, a high-threat situation. Finally, we looked at the survival rate of spiders after being pierced by sharp apophyses that \textit{M. cuspidatus} have on legs IV. The harvestmen only showed defensive behaviours in the high threat situation. Surprisingly, their mostly known defensive behaviours (chemical defence, tanathosis, pinching with chelicerae and pedipalps) were not seen even in the high threat situation. This is the first evidence that these behaviours are not used against a natural predator that has an almost 80\% predation success when attacking harvestmen. Pinching with the sharp legs IV apophyses may perforate but do not kill the spiders. We highlight the importance...
of the traditional descriptive approach with natural predators to understand the specificities of defensive behaviours against different types of predator.

Keywords
Arachnida, Laniatores, defensive behaviour, deimatic, chemoreception.

1. Introduction

According to the threat sensitive hypothesis, modulating the defensive behaviour with the level of threat is evolutionarily advantageous (Helfman, 1989), and previous studies have indeed shown that many prey react differently depending on the threat imposed: these reactions can be physiological responses of stress (Monclús et al., 2009), increased vigilance time (Mathoth et al., 2009), thanatosis (Gyssels & Stocks, 2005) and distinct behaviours according to the distance of the predator (Kleindorfer et al., 2005). Prey can exhibit a threat sensitive behaviour related to the size of the predator, for example by avoiding only larger individuals (Chivers et al., 2001). In mites, latency to lay the first egg and the total number of eggs laid can also be affected by the level of threat by the presence of predator cues as well as to the predator species (Ferrari & Schausberger, 2013). Finally, some monkeys can modulate their behaviour according to the threat imposed by human presence (Papworth et al., 2013).

When animals face a low threat like chemical cues of predators, they can avoid the site, increase locomotion, decrease movements and flee (Kats & Dill, 1998 for a review). After the predator gets closer or grasp the prey, defensive behaviours such as retaliation come into play (Edmunds, 1974). Among the weapons used to retaliate predators, some seem to have evolved from mechanisms of prey capture, others from functions related to intra-specific interactions and others are supposed to have evolved only for defensive reasons (Edmunds, 1974).

Harvestmen, arachnids belonging to the order Opiliones, have several anti-predator behaviours. When facing a low threat situation such as the presence of predator chemical cues, *Eumesossoma roweri* (Eupnoi: Sclerosomatidae) alters its locomotory pattern (Chelini et al., 2009). A presumably high threat situation such as a human attempt to hold it causes harvestmen to release defensive secretions produced by glands that open dorso-ventrally, to autotomise locomotory appendages, to shake the body, to flee, and to pinch
the potential predator with apophyses on the legs, the so called ‘nipping behaviour’ (the harvestman flexes legs IV so that the apophyses of the femur of the fourth pair of legs pinch against the body of the attacker) (Gnaspini & Hara, 2007).

Despite the great variety of defences that harvestmen possess, many animals prey upon individuals of this group. The list includes birds, frogs, opossums, hemipterans and spiders (Gnaspini & Hara, 2007). Among neotropical spiders, Ctenus fasciatus (Araneae, Ctenidae) feeds on harvestmen of the subfamily Goniosomatinae (Opiliones, Gonyleptidae) (Gnaspini, 1996; Machado et al., 2000) but there are no detailed studies on this prey–predator system. The only pairs of spiders–harvestmen that have been studied in details were spiders that do not usually eat harvestmen. These were carried out exactly with the purpose of understanding the proximal causes of rejection (Eisner et al., 2004; Souza & Willemart, 2011; Carvalho et al., 2012; Dias & Willemart, 2013; see also Machado et al., 2005). It has been shown that the hard integument is a highly important defence in these harvestmen belonging to the suborder Laniatores, and overcoming or avoiding it somehow is a prerequisite to feed on these animals. We have shown that the delicate recluse spider Loxosceles gaucho (Gertsch, 1967) (Sicariidae) bites such prey at the few vulnerable spots of the integument and is a very efficient predator of such harvestmen (Segovia et al., in press). It seemed therefore to be a good system to understand how prey react to different levels of threat by predators. In addition, we expected that most known defensive behaviours in laniatorid harvestmen would be revealed in interactions with such efficient predators. We therefore conducted a detailed behavioural analysis of the interaction between the recluse spider and the syntopic harvestman Mischonyx cuspidatus (Roewer, 1913) (Gonyleptidae). We first tested whether these harvestmen changed their locomotory behavioural pattern when exposed to chemical cues of dangerous predators (a situation of low threat). Then we looked at the defensive behaviours of the harvestmen when the spider is approaching, after spider’s touches and after spider’s bites (all situations considered to be of high threat based on data by Segovia et al., in press). Since preliminary data showed that the sharp spines of legs IV in M. cuspidatus may perforate the abdomen of a spider and because prey are expected to use their most dangerous weapons in situations of high threat, we also tested the hypothesis that such sharp spines are lethal when the abdomen of the spider is perforated.
2. Material and methods

2.1. Species studied

The spider *Loxosceles gaucho* is a predator that includes harvestmen in its diet because it is able to find and bite the weak points of the thick integument of laniatorid harvestmen (Segovia et al., in press). This spider typically builds a web sheet in which it is usually found, but it can also leave the web and capture prey out of it (Fischer et al., 2006). In laboratory experiments of predation under different substrate conditions and in the presence or absence of their web sheets, the success rate of prey capture was 77.3% with pooled data, combining substrates and presence/absence of web \( (N = 66) \) (Segovia et al., in press).

The harvestman *Mischonyx cuspidatus* has defensive behaviours that include the release of defensive secretions by the ozopores (Hara et al., 2005), pinching with sharp apophyses on legs IV (Figure 1), thanatosis by flexing the legs and keeping them close to the body (Pereira et al., 2004) and vibrating legs II, a putative defensive behaviour recently described (ILT or intense

![Figure 1. A harvestman *Mischonyx cuspidatus* (Arachnida, Opiliones) male, dorsal view. Black arrows show the sharp apophyses of the femur of the fourth pair of the legs. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1568539x.](image)
leg tapping, see Dias et al., 2014). In the field, dead carcasses of *M. cuspidatus* have been found on the webs of *Loxosceles* and theridiid spiders (Mestre & Pinto da Rocha, 2004, pers. obs.).

2.2. Collection and maintenance

*Loxosceles gaucho* was collected in a terrain located in the city of Mairiporã-SP, within a pile of bricks (23°19′S, 46°35′W). They were collected between 24 November 2012 and 26 February 2013. *Mischonyx cuspidatus* was also found in that same area, but for this study they were collected at Parque Ecológico do Tietê — São Paulo (23°25′S, 46°28′W) between 20 December 2012 and 8 May 2013. Both species co-occur in several locations (e.g., Dias & Willemart, 2013).

In the laboratory, they were both individually maintained in plastic containers (12 × 8 × 4 cm height) with soil on the bottom. Water was provided with a wet cotton ball. We fed the spiders with larvae of the tenebrionid beetle *Zophobas* sp. and nymphs of crickets (*Gryllus* sp.) according to the schedule of the experiments, the starvation period for spiders before the experiments varying between 5 and 12 days. Harvestmen were fed once a week with moistened dog food.

During the experiments, the animals were maintained in a room with a weak red light (15 W) on for 24 h per day. The natural daylight cycle was maintained and temperature was ambient. All experiments were carried out from 8:30 pm to 5 am.

2.3. Behaviour of the harvestmen in a low threat situation

This experiment was conducted to test the hypothesis that harvestmen react to chemicals left on the substrate by dangerous predators. We predicted that the harvestmen would change their locomotory behaviour in the presence of the spider chemicals (see Chelini et al., 2009). To test it, the harvestmen (*N* = 20) were exposed to filter paper in the following conditions, with one single stimulus per trial: (1) chemicals of spider; (2) a chemical control (mate tea) and (3) a blank control. Mate tea is an adequate chemical control because harvestmen do not innately prefer to stay on it or avoid it, but they are able to detect it (Santos et al., 2014). Each harvestman was tested three times in a design of repeated measures with an interval of 24 h between the tests for each individual. Individuals in all treatments were tested in distinct hours of the night.
To impregnate the filter paper with the spider cues, we maintained one adult female of *L. gaucho* (5–10 days after it received its last meal) for 24 h in a container (8 cm diameter × 10 cm height) with filter paper on the bottom. We removed the spider immediately before the test. We then removed silk deposited on the bottom with forceps, to prevent harvestmen getting stuck within it. In the chemical control group, we covered the filter paper with a thin layer of mate tea and maintained it there for 24 h, removing it right before the trials (no water was added, we only used powder). In the blank control, the filter paper was not impregnated. In each group the container was closed while impregnating (the 24 h period before the test).

Tests were run in the same container where we impregnated the filter paper with one of the chemicals (or control with no cues). We placed the filter paper on the bottom and a neutral central disc of filter paper (4 cm diameter) in the centre. We first acclimated the harvestman in a vial (4 cm diameter × 7.5 cm height) placed in the centre of the arena (in the neutral disk). After two min, the harvestman was released and we recorded it for 20 min with a Sony HDXR550V Handycam in nightshot mode. The harvestmen were submitted to each of the three treatments in a systematic alternated order: the sequence of treatments was such that no sequence was more common than others and there was no bias concerning the hour of the day and the treatment.

We compared three behavioural categories between the treatments: the time harvestmen spent: 1, stationary (motionless); 2, stand waving (stationary but leg waving); and 3, walking (displacing around the arena) (cf., Chelini et al., 2009). We used a Friedman test (because the data did not follow a normal distribution), followed by a post-hoc Student–Neuman–Keuls test.

2.4. Behaviour of the harvestmen in a high threat situation

To describe the behaviour of the harvestmen in a high threat situation, we reanalysed videos used in an experiment for another project, which was conducted to test, from the spider point of view, the role of silk on predation (Segovia et al., in press). Spiders in both the “no silk” group (*N* = 19, 17 females and 2 males) and the “silk group” (*N* = 19, 13 females and 6 males) were offered one harvestman each. We used 10 females and 9 male harvestmen per group (38 harvestmen used in total). Twenty five to 29 days before the trials (following Souza & Willemart, 2011), we offered one cricket nymph (*Gryllus* sp., ≈1.5 cm) and one beetle larvae (*Zophobas* sp.)
simultaneously to the spiders, to standardize hunger. Only spiders that ate at least one prey were tested. Prey uneaten after 48 h were removed. The terraria of the experiments (18 cm diameter × 6 cm height) were the same used to maintain the spiders. During the tests we covered the terraria with a sheet of glass to prevent the spiders from fleeing. Spiders in the treatment group were simply removed and put back in their terrarium. For spiders in the control group, we took them out of the terrarium, we removed all the soil, cleaned the whole terrarium with a dry paper towel to remove remnants of silk and then put new soil in it. This was done because removing silk was not possible without removing the soil. We then reintroduced the spider. This procedure did not affect the behaviour of the spider, as shown elsewhere (Segovia et al., in press). In both groups, we allowed the spider to walk freely in the terrarium for 15 min before we introduced a harvestman as far as possible of the spider.

We recorded the experiments using a Sony HDXR550V Handycam in nightshot mode. We recorded for 40 min after the first bite. Each spider was tested only once. We collected the data between 21 February and 7 March 2013, between 9 pm and 5 am. We used both the videos with the presence and absence of silk because the predation success between the two treatments was the same (Segovia et al., in press). We only analysed videos in which the spider bit the harvestman. The behavioural analysis started about 5 s before the spider touched the harvestman and finished 2 min after the first bite by the spider. We looked at the behavioural categories displayed by the harvestmen. Preliminary analyses of ten videos (5 silk group and 5 no-silk group) showed that increasing this time did not result in more behavioural categories observed.

2.5. Potential lethality of a harvestman defence under high threat

Nipping behaviour has been observed in interactions between *M. cuspidatus* and the spiders *Ctenus ornatus* (Keyserling, 1891) (Ctenidae) and *L. gaucho* (Dias & Willemart, 2013; this paper). This behaviour is potentially dangerous for a predator since *M. cuspidatus* has a sharp apophysis on the retrolateral portion of the femur of both legs IV. When they rapidly flex their legs at the coxa-trochanter articulation (= ‘nipping’), the two apophyses pinch what is in between the legs. By experimentally holding the spider by the prosoma and allowing a male *M. cuspidatus* to naturally pinch the abdomen of the spider with these apophyses, we found that the harvestman
is capable of perforating the abdomen of *L. gaucho*, which loses hemolymph (Figure 2). Because losing hemolymph seem to potentially cause death in another harvestman (e.g., Willemart, 2002), we tested the hypothesis that a perforation caused by these apophyses negatively affects the survivorship of the spiders *L. gaucho*. We predicted that perforated spiders would have a lower survival rate than the spiders that were not perforated.

We used two groups in this experiment. In the treatment group (*N* = 10), we anesthetised the spiders with CO₂ by having them in a closed vial with a small aperture on the top in which the gas was injected for 1.5 min. We then held the spider within the femora of a male adult harvestman so that we could manually flex its legs, pinch and perforate the spider’s abdomen. We used a different harvestman for each spider and the same person held the harvestmen in every test. In the control group (*N* = 10), we used the same procedures, but we pinched the abdomen with a cushioned forceps,
again always by the same person. The experiment was carried out in May 2013 between 8 pm and 3 pm. The harvestmen were then maintained in plastic containers (12 × 8 × 4 cm height) with soil on the bottom. Water was provided with a wet cotton ball. In the 5 subsequent days, we verified the survivorship of the spiders between 10 and 11 pm and after 40 days we monitored again. We compared the survival rate at the end of the experiment using a $\chi^2$ test.

3. Results

3.1. Behaviour of the harvestman in a low threat situation

There were no differences when comparing the controls (blank and mate tea) and the treatment group (spider chemicals) in the time that the harvestman spent: stationary ($p = 0.071$), stand waving ($p = 0.170$) or walking ($p = 0.744$). The animals spent more time stand waving in all groups ($p < 0.05$): mate tea, blank and spider chemicals (Table 1). No defensive behaviour was observed.

3.2. Behaviour of the harvestman in a high threat situation

We found behaviours that can be considered potentially defensive in *M. cuspidatus* in different phases, as detailed below (see Table 2 for descriptions). The results are divided in ‘no-silk group’ ($N = 15$) and ‘silk group’ ($N = 17$). Sample sizes provided below are the number of animals that have shown each behaviour.

| Table 1. |
| Time spent by the harvestman *Mischonyx cuspidatus* in three behavioural categories, in three different treatments: filter paper without stimuli; filter paper with mate tea (chemical control) or filter paper with predator chemicals. |

<table>
<thead>
<tr>
<th>Behavioural category/treatment</th>
<th>Time spent (s) (median; MIN–MAX)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blank</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Stationary</td>
<td>49; 0–1087</td>
</tr>
<tr>
<td>Stand waving</td>
<td>903; 0–1200</td>
</tr>
<tr>
<td>Walking</td>
<td>23; 0–452</td>
</tr>
</tbody>
</table>

The harvestmen were recorded for 1200 s. Each treatment was tested separately. MIN, minimum; MAX, maximum.
Table 2.
Behavioural repertory of the harvestman *Mischonyx cuspidatus* when interacting with the recluse spider *Loxosceles gauch*, based on 32 predatory events.

<table>
<thead>
<tr>
<th>Behavioural category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intense leg tapping (ILT)</td>
<td>Intense dorso-ventral movements of legs II</td>
</tr>
<tr>
<td>Motionless</td>
<td>Not moving the body or the locomotory appendages</td>
</tr>
<tr>
<td>Move away from the spider</td>
<td>The harvestman walks or try to walk away from the spider</td>
</tr>
<tr>
<td>Move toward the spider</td>
<td>The harvestman walks or tries to walk toward the spider, often when being held by the spider</td>
</tr>
<tr>
<td>Move pedipalps</td>
<td>The harvestman displays dorso-ventral movements with the pedipalps</td>
</tr>
<tr>
<td>Nipping</td>
<td>Pinching with legs IV that bear sharp apophyses</td>
</tr>
<tr>
<td>Pull the leg</td>
<td>The harvestman quickly moves its legs dorso-ventrally, probably trying to get rid of silk threads</td>
</tr>
<tr>
<td>Self-grooming</td>
<td>The harvestman passes legs and or pedipalps between the chelicerae</td>
</tr>
</tbody>
</table>

Phase 1, pre-contact: In the no-silk group, we did not find behaviours considered as defensive. In the silk group, we have observed the behaviour ‘pull the leg’ \( (N = 6) \).

Phase 2, post-contact, before biting: In the no-silk group, we have observed ‘move away from the spider’ \( (N = 3) \); ‘motionless’ \( (N = 2) \); ‘ILT’ \( (N = 1) \) and ‘move toward the spider’ \( (N = 1) \). In the silk group we have observed ‘motionless’ \( (N = 4) \); ‘ILT’ \( (N = 1) \); ‘move away from the spider’ \( (N = 1) \) and ‘move pedipalps’ \( (N = 1) \).

Phase 3, post-contact, after biting: In the no-silk group we have observed ‘move toward the spider’ \( (N = 13) \); ‘move away from the spider’ \( (N = 8) \); ‘motionless’ \( (N = 2) \); ‘move pedipalps’ \( (N = 1) \); ‘ILT’ \( (N = 1) \) and ‘nipping’ \( (N = 1) \). In the silk group, we have observed ‘pull the leg’ \( (N = 9) \); ‘motionless’ \( (N = 2) \); ‘move away from the spider’ \( (N = 2) \) and ‘nipping’ \( (N = 1) \). We did not observe the release of the defensive secretions, thanatosis, and pinching with pedipalps and chelicerae in any of the phases analysed.

3.3. Potential lethality of a harvestman defence under high threat

One individual died in the control group in the first day and one died in the treatment group in the second day. All the others stayed alive until the end of the 5 days in both treatments. There were no differences in the survival rate after 40 days \( (\chi^2 = 0.00, \text{df} = 1, p = 1.00) \).
4. Discussion

We observed defensive behaviours in *M. cuspidatus* in a high threat situation but not in a low threat situation, in which we also did not detect differences in the time the harvestmen spent stand waving, walking or stationary between treatments. Perforation of the spider’s body by the harvestman did not affect its survival.

Because no defensive behaviour at all could be noticed in the presence of predator chemicals, this is partially in accordance with previous studies that show prey changing its behaviour according to the level of threat (Sih et al., 1986; Bothan et al., 2008; Walzer & Schausberger, 2011). An example in arachnids is the spider *Latrodectus hesperus* (Theridiidae) that can modulate its defensive bites, doing it more frequently in a high threat situation than in medium and low threat (Nelsen et al., 2014). But the lack of reaction of *M. cuspidatus* to chemical cues of *L. gaucho* contrasts with the results obtained for spiders (e.g., Persons et al., 2001, 2002; Wilder & Rypstra, 2004) and for the harvestman *Eumesosoma roeweri* (Chelini et al., 2009) that exhibited defensive behaviours in the presence of chemicals of the spider *Schizocosa ocreata* (Lycosidae) (Chelini et al., 2009). A possible explanation for this lack of defensive behaviour in a low threat situation is that the harvestmen did not detect the spider chemicals, but that seems unlikely since laniatorids have chemoreceptors in the first and second pair of legs and are capable of detecting chemicals at close range or contact (Willemart & Chelini, 2007; Willemart et al., 2009; Costa & Willemart, 2013). An alternative hypothesis is that harvestmen do not avoid the chemicals because they cannot determine the spider species only by their chemicals. Indeed, most syntopic spiders are not efficient predators of *M. cuspidatus*, and unnecessary predator avoidance may result in less weight gain (Chelini et al., 2009). Moreover, the hard integument plays a great role in defence in species of the suborder Laniatores (Souza & Willemart, 2011; Carvalho et al., 2012; Dias & Willemart, 2014; Segovia et al., in press). The soft integument in Eupnoi could partially explain why *E. roeweri* show defensive behaviours in the presence of predator chemicals only (Chelini et al., 2009), but *M. cuspidatus* (with a hard protective integument) do not. In any case, it should be considered that prey perform defensive behaviours more often towards specialists than towards generalist predators (Crawford et al., 2012), such as *Loxoseles* spiders (Fischer et al., 2006).
In the presence of the spider, in the pre-contact phase, we did not detect any defensive behaviour in the ‘no-silk group’. Only in the silk group we observed the behaviour ‘pull the leg’, suggesting it is related to attempts of getting rid of the silk. Moving away from the spider is probably an attempt to escape, one of the most common defensive behaviours among animals (Edmunds, 1974). We also observed ILT (intense leg tapping), a behaviour first described in male–male fights (Willemart et al., 2009) and then in a defensive context (Dias et al., 2014). In our study, in one case *L. gaucho* stopped the attack temporarily after this behaviour and in the other case the spider was touching the leg of *M. cuspidatus* and did not change its behaviour after ILT.

In the phase 3 (post-contact after biting), the most common behaviours we have observed were ‘move toward the spider’ and ‘move away from the spider’. We have also observed ‘motionless’, which is expected to be important against spiders since these animals typically rely also on vibratory cues to detect prey (Barth, 2002). But the recluse spiders attacked in all cases after the harvestmen displayed this behaviour, which is in accordance with our data that vibratory cues may not be necessary for *L. gaucho* at least for capturing harvestmen without silk (Segovia et al., in press). Further studies should be conducted to test which sensory modality is mainly used by these spiders to detect their prey. Visual stimuli are known to be used for prey capture by spiders not known for their visual acuity such as ctenids (Pirhofer et al., 2007; Fenk et al., 2010). Air displacements detected by trichobothria are another possibility (Barth, 2002).

Nipping behaviour has long being cited as an anti-predator behaviour (see references in Gnaspini & Hara, 2007), but only recently it has been observed against a natural predator (Dias & Willemart, 2013). Now we have shown that it has the potential to pierce the body of a predator so it may not only be deimatic, since losing haemolymph can be costly. At least in males of some crickets, the loss of haemolymph can decrease reproductive success (Sakaluk et al., 2004). Nonetheless, we do not know if the amount of haemolymph lost by the spiders in our study is enough to affect reproduction. Although our results provided no evidence of survival costs, in nature such injuries could affect spider behaviour or reduce the spider’s survivorship since animals are more exposed to pathogens, and therefore open wounds can act as entry for bacteria (Loof et al., 2010).
Some of the mostly known defences of laniatorids are the release of repugnatory chemicals and mechanical retaliation such as pinching with chelicerae and pedipalps (Gnaspini & Hara, 2007). *Mischonyx cuspidatus* in particular is also known for displaying thanatosis for several minutes, bringing the legs close to the body. This is the first evidence that none of these behaviours are observed against such a successful predator (almost 4 in each 5 attacking *L. gaucho* successfully feed on *M. cuspidatus*), and typically not against other spiders too (Souza & Willemart, 2011; Carvalho et al., 2012; Dias & Willemart, 2013). Since most descriptions of chemical defences in harvestmen were made based on human manipulation (see references in Gnaspini & Hara, 2007), chemicals may be mainly used against vertebrate predators at least in some species that are more reluctant to release secretions, such as *M. cuspidatus*. Some anurans with toxic glands flex their legs when being attacked and swallowed by predators, which often regurgitate them. Flexing the legs may be a way to minimize the chances of injuries to the legs (see Toledo et al., 2010). The same could happen in *M. cuspidatus*, but it has never been tested against any natural vertebrate predator. At least the repugnatory chemicals of one laniatorid have been tested and proved to be effective against toads, but not opossums (Machado et al., 2005). A domestic dog has also been observed to be deterred by the secretions of *Acanthogonyleptes fulvigranulatus* (Gonyleptidae) (G.F. Pagoti, pers. commun.). After the dog sniffed on the harvestman, it released secretions on the dog’s nose. The dog subsequently rubbed its nose against the substrate and did not chase the harvestman. Since the dog only sniffed and did not bite, the possibility that CO2 from the predator influences the release of defensive secretions remains to be tested.

We have shown that some of the most known defences of laniatorid harvestmen such as releasing repugnatorial chemicals, pinching with chelicerae and pedipalps and thanatosis are surprisingly not used against a very dangerous spider. Studies with arthropod defence and specifically chemical defences follow a tradition of manipulating with tweezers or by hand (e.g., Eisner et al., 2005). Great advances were made, but several defensive behaviours seen against tweezers or human hands are not used against some natural predators. Such behaviours are probably specific to some predators. A clear methodological message of this and previous studies (Souza & Willemart, 2011; Carvalho et al., 2012; Cook et al., 2013; Dias & Willemart, 2013)
is that ethologists have to be careful when designing experiments of prey–
predator interactions. For example, knowing that a species releases defensive
secretions does not mean it will release them against any predator. Testing
the effect of secretions on predators against which secretions are not used is
unnatural, and this also applies to the huge number of arthropods and other
animals that use chemical defences. The same applies to non-chemical de-
fences. Finally, the traditional descriptive approach is crucial to unravel such
specificities. As noted by Tinbergen (1963), “We must hope that the descrip-
tive phase is not going to come to a premature ending”.

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avoidance by slimy sculpins: understanding the importance of visual versus chemical


Defensive behaviour of a harvestman against a spider


