Harvest-ironman: heavy armature, and not its defensive secretions, protects a harvestman against a spider

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Natural selection has caused prey species to evolve distinct defensive mechanisms. One of such mechanisms was the evolution of noxious or distasteful chemicals, which have appeared independently in a number of vertebrates and invertebrates. In detailed analyses of arthropod behaviour, scent gland secretions have consistently been shown to be responsible for repelling specific predators. Because using such chemicals is costly, animals with alternative cheaper defences are expected not to release such secretions when alternative options exist. In this study, we sought to determine the defensive mechanisms of the harvestman *Discocyrtus invalidus*, a heavy bodied species that bears a pair of repugnatorial glands. The spider *Enoploctenus cyclothorax* was used as the predator, and the cricket *Gryllus* sp. was used as a control. In a first set of experiments, the harvestmen were preyed upon significantly less than the crickets. In two other experiments, we found that harvestmen did not use their scent gland secretions to deter the predator. Moreover, results of a fourth experiment revealed that these spiders are not repelled by defensive secretions. *Discocyrtus invalidus* has a thick cuticle on the entire body: scanning electron micrographs revealed that only the mouth, the articulations of appendages and the tips of the legs are not covered by a hard integument. In a fifth experiment, we found that these spiders had difficulty piercing the harvestmen body. This is the first experimental evidence that a chemically defended arachnid does not use its scent gland secretions to repel a much larger predator but instead relies on its heavily built body.

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Selective pressures caused by predators on prey species have led to the appearance of distinct defensive mechanisms that favour greater survival and consequently a greater probability of generating offspring (Lind & Cresswell 2005). In chemical defence, noxious or distasteful chemicals have appeared independently several times within metazoans, having evolved among sponges, cnidarians, molluscans, annelids, arthropods, echinoderms, ascidians and vertebrates such as amphibians, reptiles, mammals and birds (McClintock & Baker 1997; Dumbacher et al. 2000; Pisut & Pawlik 2002; Wood et al. 2002; Brizzi & Corti 2007; Hutchinson et al. 2007; Meredith et al. 2007; Hanifin et al. 2008; Fleury et al. 2008; Tschuch et al. 2008; Peters et al. 2009; Sheybani et al. 2009). Such chemicals can cause the predator to simply release the prey but in some cases may even cause temporary paralysis or death (e.g. Carrel & Eisner 1984; Hagman et al. 2009; Hayes et al. 2009).

Arthropods have a particularly interesting array of repellent chemicals (Roth & Eisner 1962). The identification of their scent gland secretions and their repelling effects on predators have been studied in different taxa, including Myriapoda, Insecta and Arachnida (Eisner 1970, 2005). Arachnids often release their prey and may even autotomize their own legs in an attempt to get rid of the secretions (Eisner 2005; Eisner et al. 2006). However, the costly production of defensive chemicals (McCormick & Larson 2008) could affect development, resulting in a reduction in adult size (Cohen 1985; Rowell-Rahier & Pasteels 1986; Dobler & Rowell-Rahier 1994; Zalucki et al. 2001) and lower mating success (Andersson 1994). Therefore, animals with defences that would not require the costly replenishment of scent glands are expected to avoid using their secretions unless it is absolutely necessary.

Harvestmen (Arachnida, Opiliones) have defensive mechanisms such as fleeing, feigning death, pinching with chelicerae, pedipalps or leg spines and autotomizing legs (Gnaspini & Hara 2007). They also possess a pair of scent glands that open in the lateral regions of the cephalothorax (Gnaspini & Hara 2007). When disturbed, harvestmen may release water from their mouth that eventually mixes with the scent gland secretions (see Eisner et al. 2004). This increases the amount of liquid used as a repellent. Not

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only are harvestmen chemically defended, but they also have a hard integument that could be protective just like the carapaces that have convergently evolved among mammals (armadillos and pangolins; Nowak 1999), reptiles (turtles; Pritchard 1979) and other arthropods (beetles and mites; Sanders & Norton 2004; Majerus et al. 2007). Although several harvestmen species, such as cyphophthalmids and laniatorids, and some Dyspnoi (van der Hammen 1985; Shultz & Pinto-da-Rocha 2007) have a strong cuticle, it is unknown whether this cuticle offers protection against predators.

In the present study, we conducted a detailed behavioural analysis of the interaction between the harvestman *Discocyrtus invalidus* (Opiliones, Gonyleptidae) and the syntopic generalist spider *Enoploctenus cyclothorax* (Araneae, Ctenidae). We first tested whether the spider preyed upon the harvestmen, then explored the details of their behaviour. Next, we experimentally tested whether the release of small amounts of scent gland secretions, invisible to the human eye, could play a role in protecting the harvestmen. We then experimentally tested the effect of the scent gland secretions per se on the spider, and finally tested the possible role of the heavily built body of the harvestman in defence. Taken together, we present a case in which, in accordance with the notion of the economical use of costly resources such as gland secretions (see e.g. Nolen & Johnson 2001; Radwan et al. 2006), the scent gland secretions are not used to repel a predator even after an attack: *D. invalidus* instead seems to rely on its strong cuticle for protection against the much larger *E. cyclothorax*.

METHODS

Species Studied

The harvestman *Discocyrtus invalidus* (Piza 1938) (Laniatores, Gonyleptidae) is found in forests, where it hides under logs during the day and wanders on tree trunks, on the ground or on bushes at night. The scent gland secretions of these animals are composed mainly of highly volatile 2,3-dimetil-1,4-benzoquinone (Hara et al. 2005). Their natural predators are not known.

The spider *Enoploctenus cyclothorax* (Bertkau 1880) (Ctenidae) is a large spider that hides beneath palm sheats and in natural holes in forests during the day and sits and waits for prey at night. They feed on a variety of arthropods, such as large roaches and crickets, other spiders (including conspecifics) and isopods (Willemart & Kaneto 2004). Prior to this study, it was unknown whether *E. cyclothorax* preys on *D. invalidus*.

Collection and Maintenance in the Laboratory

Adult harvestmen and adult and subadult spiders were collected at night, at the 'Reserva da Cidade Universitária (C.U.A.S.O)' and 'Parque Esporte Para Todos' both at the Cidade Universitária Armando de Sales Oliveira, São Paulo city, State of SP, Southeastern Brazil (23°32′51″ S, 46°38′10″W). The animals were numbered and maintained in individual plastic containers ($12 \times 8 \times 4$ cm for harvestmen; 20 cm diameter $\times$ 8 cm height for spiders). The bottom was covered with humid soil and water was provided by a wet cotton ball. Harvestmen fed once a week on moistened dog food and *Tenebrio molitor* beetle larvae, and spiders were fed on crickets once a week except when experiments were being conducted. The Chico Mendes Institute provided licences for collection and maintenance of animals in the laboratory. After the study, some animals were fixed in ethanol 70% and others were released at the same site where they had been collected.

Experiments

We ran the trials of experiments 1–4, from April to October 2009, under dim light, at room temperature (20–25 °C). Data from experiments 2–4 were collected between 1800 and 2400 hours. Experiment 5 was conducted in March 2010, at room temperature, normal light, and between 2200 and 0200 hours. We used a Sony Handycam DCR-TRV361 'nightshot'. For experiments 1–4, the proportion of subadults (last stage before adulthood) and adults and of males and females were equally distributed among treatments. Only three adult males of *E. cyclothorax* were used. Adult females are usually slightly larger than subadults. Adult males have longer legs. Except for this criterion, the animals were randomly distributed among treatments.

Experiment 1

To verify whether *E. cyclothorax* prey on *D. invalidus*, one individual of each species was left for 5 days in the same arena (20 cm diameter $\times$ 8 cm height) ($N = 16$). As a control, another 16 spiders were left with crickets for 5 days. All 32 spiders were starved for 25–30 days before day 1 of the experiment to ensure that they would be hungry and therefore motivated to eat. We monitored all containers for predation daily between 1200 and 1300 hours and then compared the predation rate between control and experimental treatments after the fifth day.

Experiment 2

In experiment 2, we were interested in observing the details of the predatory interaction. Thirty two spiders were exposed to either a harvestman or a cricket ($N = 16$). All 32 spiders were starved for 25–30 days before day 1 of the experiment. The circular arena used for the tests (20 cm diameter $\times$ 8 cm height) had humid soil on the bottom. The spider was introduced into this arena 8 h before the trial to minimize stress, and the harvestman was introduced in a vial as far as possible from the spider, allowed to acclimate for 2 min and then released. By digitally recording the trials, we recorded behaviours related to the approach between the two animals, the physical interaction and subsequent 10 s, which was sufficient to detect whether the spider would start eating the prey. From the videos, we created and quantified behavioural categories and compared their occurrences across treatments.

Experiment 3

In experiment 3, we tested the hypothesis that harvestmen can secrete small amounts of defensive secretions, invisible to the human eye. This would form a chemical shield that would explain why *E. cyclothorax* would reject *D. invalidus*. Forty eight spiders were subjected to one of four treatments ($N = 12$ per treatment): (1) harvestmen with gland experimentally obstructed with glue; (2) harvestmen with glue on the dorsum; (3) crickets with glue on the dorsum; (4) crickets with no glue. The glue was applied at least 15 days before the trial to minimize the possibility of having residual odours. The remaining methodological procedures were the same as in experiment 2. From the videos, we quantified behavioural categories and compared their occurrences across treatments.

Experiment 4

Most laniatorids use evasive defensive behaviours (e.g. fleeing) or aggressive mechanical defensive behaviours (e.g. pinching with the pedipalps and chelicerae, or with the spines on the fourth pair of legs; Gnaspini & Hara 2007). Therefore, testing the isolated effect of the chemicals from their scent glands required using a design where the harvestman was absent. We collected the exact content of the scent glands released by 10 harvestmen, including secretions
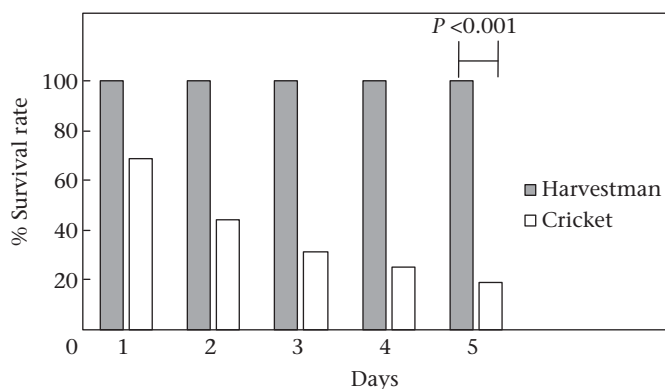
Figure 1. Survival rate of crickets (*Gryllus* sp.) and harvestmen *Discocyrtus invalidus* when paired with *Enoploctenus cyclothorax* for 5 days.

Figure 2. *Enoploctenus cyclothorax* 'ignoring' (as described in the ethogram in Table 1) the harvestman *Discocyrtus invalidus*. Scale bar = 1 cm.

from the scent glands and water released from the mouth (5 μl in total), using a microsyringe (F-GT SGE) while we held each individual. Following Hara et al. (2005), we collected secretions on previous days and kept them in the freezer until their use the following day, at which time we allowed the secretions to thaw completely before use. This methodology does not affect the odours (see Hara et al. 2005). During the trials, we offered a cricket to a spider, and immediately following the spider's capture of the cricket, we applied the secretions between the spider's chelicerae using a syringe (cf. Eisner et al. 1997) ($N = 10$). Using a repeated measures design, we adopted the same procedure for controls except we applied distilled water between the spider's chelicerae instead of secretions. The order in which the individuals were used and the order of treatments were established at random. Following application of the liquid, we monitored the animals for 10 min and recorded whether the cricket was released in each trial.

Experiment 5

To test the efficiency of the hard integument of *D. invalidus*, we collected 10 *E. cyclothorax* (4 subadult males, 5 adult females, 1 subadult female) and 10 *D. invalidus* (6 adult males, 4 adult females). We held the spider between our fingers, gently pressing its prosoma against the edge of a table, and positioned a harvestman between the spider's chelicerae. Eventually, the spider would bite the harvestman. We digitally recorded the trials and later analysed videos and pictures produced with the software VirtualDub (http://www.virtualdub.org/).

Morphological Features

We cleaned the focal body region ultrasonically, then dried it in a stove at 40 °C for at least 24 h, mounted on an aluminium stub using double-stick adhesive tape, sputter coated with gold (Sputter Coater Balzer SCD 50), and photographed with a Zeiss DSM 940 scanning electron microscope.

RESULTS

Experiment 1

Thirty per cent of the crickets were preyed upon within 1 h after being offered to the spiders. All harvestmen were alive by the end of the 5-day period, whereas less than 25% of the crickets remained alive ($\chi^2_1 = 18.656$, $P < 0.001$; Fig. 1). No injuries were noted among harvestmen.
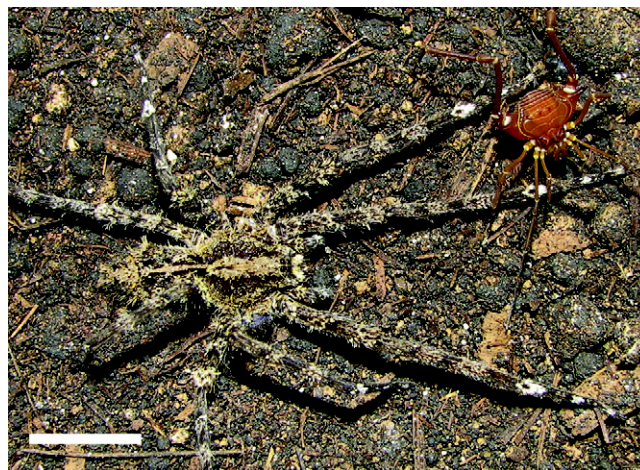
Experiment 2

Eighty one per cent of the crickets were consumed. In contrast, 81% of the spiders also attacked the harvestmen but ignored them thereafter (Fig. 2). Of the 13 harvestmen that were attacked, seven walked away from the spiders, five remained stationary and one was consumed. The only spider that consumed the harvestman touched the harvestmen's body (and not its legs) first. Defensive behaviours like tanathosis, pinching with chelicerae and pedipalps, or nippings (pinching with spines on legs IV) were not observed. In none of the observations could we see the release of scent gland secretions, nor smell it. The behavioural repertoire of the spider attack is shown in the ethogram (Table 1).

Only 3 of 16 spiders did not consume the crickets after contact. The comparative sequence of behaviours shown by spiders when attacking harvestmen and crickets can be seen in Fig. 3. Whereas crickets were mainly bitten on the body and held for ingestion, harvestmen were usually bitten on the leg, then released and ignored.

Experiment 3

Harvestmen with the glands experimentally clogged or left opened were attacked equally often (Fisher's exact test: $P = 1$; Fig. 4). As in experiment 2, no mechanical defensive behaviour was

Table 1
Behavioural repertoire of the spider *Enoploctenus cyclothorax* when interacting with the harvestman *Discocyrtus invalidus* and the cricket *Gryllus* sp.

Category	Definition
Active contact	Spider slowly moves its leg and touches the prey with the distal portion
Approach	Spider slowly moves towards the prey
Attack	Spider rapidly moves towards the prey
Desist	Spider rapidly moves back to its original position and posture after attacking
Hold	Spider holds the prey between its chelicerae for at least 10 s
Ignore	Spider remains stationary
Move away	Spider slowly walks away from the prey
Passive contact	While walking, a prey touches a motionless spider
Pinch body	Spider pinches the prey's body with its cheliceral fangs
Pinch leg	Spider pinches the prey's legs with its cheliceral fangs
Release	Spider opens chelicerae, letting go of the prey
Rotate	Spider rotates the body without displacement, ending with the anterior portion of the body facing the prey
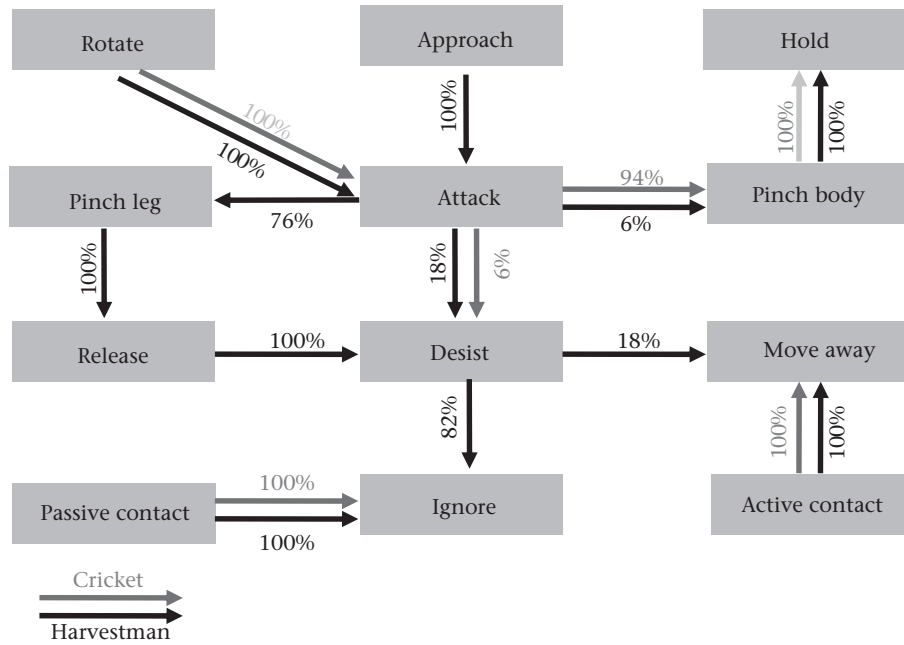
Figure 3. Combined fluxogram of the predatory interaction between the spider *Enoploctenus cyclothorax*, the harvestman *Discocyrtus invalidus* and the cricket *Gryllus* sp. Black arrows indicate acts displayed by spiders interacting with harvestmen, and grey indicates acts displayed by spiders with crickets ($N = 40$). Because we found no difference in the behaviours presented in experiments 2 and 3, this fluxogram combines results from both ($N = 40$).

displayed by the harvestman, which walked away (75%) or remained motionless after contact (21%). One harvestman with the glands clogged was consumed. Crickets of both groups were preyed upon equally (Fisher's exact test: $P = 0.398$), demonstrating that the glue had no effect on the spiders. In accordance with the results obtained in the other experiments, there was a significant difference between the harvestmen and cricket treatments ($\chi^2_3 = 26.05$, $P < 0.001$).

Experiment 4

None of the spiders released the crickets after we applied a droplet of defensive secretion or distilled water between their chelicerae ($N = 10$).

Experiment 5

Only 1 of 10 spiders pierced the body of the harvestmen. Dorsal scutum, lateral and ventral regions of coxae IV and the dorsal region of free tergites were repeatedly and unsuccessfully bitten by the spiders. In the only case where the integument was pierced, an adult female spider bit the lateral and dorsal regions of an adult male harvestman so that the chelicerae had good anchorage and did not slide. All harvestmen except the one that was pierced were alive after 1 week.

Morphological Features

Scanning electron micrographs showed the hard integument of some harvestmen species. Heavy sclerotization was present in the
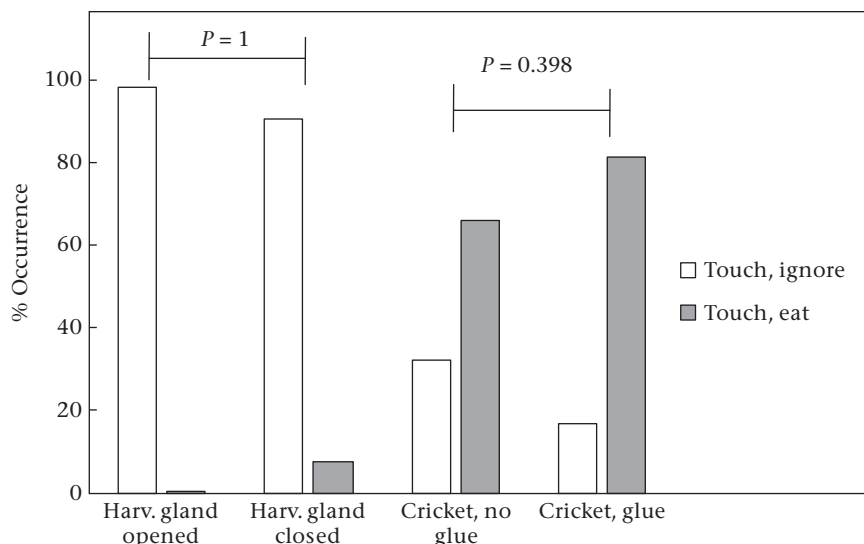
Figure 4. Behaviour of the spider *Enoploctenus cyclothorax* when interacting with crickets (*Gryllus* sp.) with or without glue on the dorsum (control groups) and harvestmen *Discocyrtus invalidus* with glue on the dorsum (control group) and glue clogging the scent gland opening (treatment group). Harv. = harvestman.
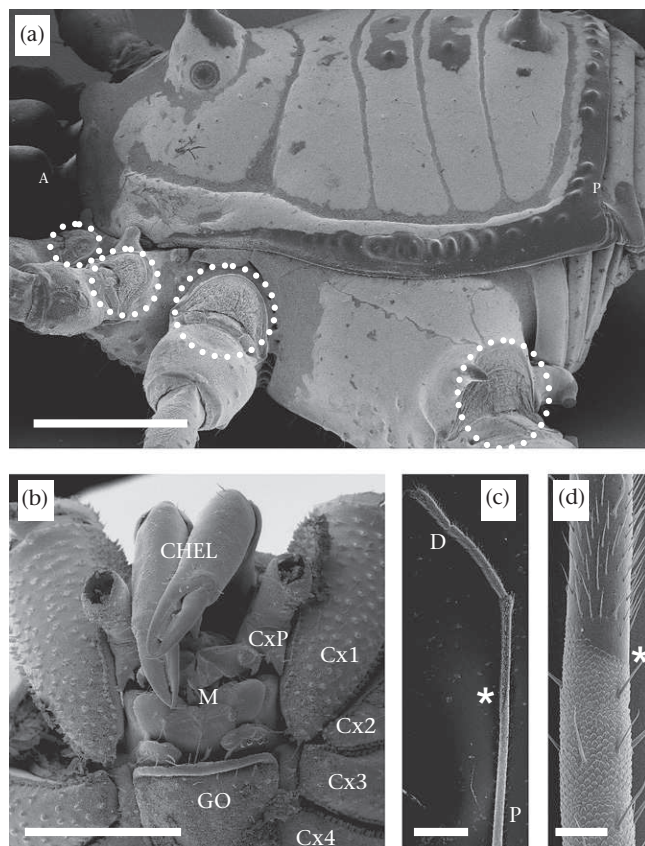
Figure 5. The hard integument of some harvestmen species: (a) *Camarana flavipalpi* (Gonyleptidae), dorso–lateral view. Dotted circles show the articulations of coxae–trochanter, areas where the integument is softer and vulnerable to predators. A = anterior; P = posterior. Scale bar = 1 mm. (b) Unidentified Gagrellinae (Sclerosomatidae), ventral region. Chel = chelicerae; C×P = coxa pedipalp; C×1 = coxa of leg I; C×2 = coxa of leg II; C×3 = coxa of leg III; C×4 = coxa of leg IV; M = mouth; GO = genital operculum. Scale bar = 150 μm. (c) *Daguerreia inermis* (Gonyleptidae), leg I, lateral view. Asterisk shows the boundary between the soft and sclerotized regions of the leg (astragalus–calcaneus). P = proximal; D = distal. Scale bar = 1 mm. (d) *Promitobates ornatus* (Gonyleptidae), leg I, lateral view. Asterisk shows the boundary between the soft and sclerotized regions of the leg (astragalus–calcaneus). Scale bar = 100 μm.

dorsal, lateral and ventral regions and also on the legs (Fig. 5). From the middle region of the metatarsus and distally, the harvestman leg was not covered by a hard integument, but was instead covered with sensory setae and trichomes (Fig. 5c, d). Therefore, the only soft regions of the body in such species are the mouth, the articulations of appendages and the tips of the legs.

DISCUSSION

Discocyrtus invalidus is not preyed upon by *E. cyclothorax* and does not release scent gland secretions when attacked. These secretions play no role in causing the rejection, and the spider is not affected by these secretions when it comes in contact with them. Scanning electron micrographs showed that there are only a few body regions that are not sclerotized and these were never bitten by the spider. *Enoploctenus cyclothorax*, despite being much larger, seldom managed to perforate the hard integument of the harvestman.

Previous studies had shown that *E. cyclothorax* rejects some harvestmen species (see references in Machado et al. 2005), but there had been no detailed investigation of the proximate mechanisms behind such rejection. We have shown that *D. invalidus* is

ignored by the spider *E. cyclothorax*, and that, surprisingly, the secretions of the defensive glands are not needed to repel the spider. This is in contrast to most studies on chemical defence in arthropods, which consistently show that chemicals from defensive glands are responsible for the rejection (e.g. Eisner & Aneshansley 2000b; Sugeno & Matsuda 2002; Machado et al. 2005; Eisner et al. 2006). Pairing *D. invalidus* with a spider predator revealed that they did not use any of their typical defensive behaviours (pinching with chelicerae and pedipalps or with spines on legs IV of males; Gnaspini & Hara 2007) or chemical defences to deter the spider, even when the spider bit them.

The scent gland secretions of *D. invalidus* did not cause *E. cyclothorax* to release the cricket from its chelicerae. Secretions of the harvestman *Acanthopachylus aculeatus* are also ignored by the spider *Lycosa ceratiola* (Lycosidae) (Eisner et al. 2004). In contrast, *Trechalea biocellata* (Trechaleidae) and *E. cyclothorax* released captured crickets after application of *Acutisoma longipes* (Gonyleptidae) secretions (Machado et al. 2005). All these harvestmen are able to produce 2,3-dimethyl-1,4-benzoquinone showing that similar chemicals may have different effects on distinct predators (see e.g. Bryana et al. 1997; López-Legentil et al. 2006). Moreover, previous studies used different volumes of secretions. Further studies should determine the threshold concentration of scent gland secretion required to have a repelling effect in different spider species.

Our results from experiment 5 suggest that *E. cyclothorax*, despite being much larger, have difficulty piercing the heavy built body of *D. invalidus*. In experiments 2 and 3, harvestmen were often bitten in proximal regions of the legs but were left unharmed, which is in accordance with our morphological data: only the mouth, the tips of the legs and the articulations are soft-bodied and probably vulnerable. The fluxogram generated by our data also showed an interesting pattern: when a harvestmen was bitten by a spider, it was most often bitten on the leg (followed by rejection), whereas crickets were fatally bitten in the head, thorax or abdomen. Because the harvestman walks with the body close to the substrate, the legs form a fence around the body, which appears to increase protection to the vital organs.

Since hard sclerotization is a widespread phenomenon in Opiliones (see Pinto-da-Rocha et al. 2007), this mechanical defence is probably efficient in several species and has probably been over-looked previously. In previous papers on spider–harvestmen predatory interactions, the explanations as to why some spiders rejected harvestmen were based on some kind of chemical defence (Eisner et al. 2004; Machado et al. 2005; Willemart & Pellegatti-Franco 2006). By clogging the scent gland secretions, we now have evidence to reject the hypothesis that scent gland secretions play a role in this specific interaction, and we suspect this might be the case in several heavy bodied species of harvestmen. Pairing salticids (Araneae) with syntopic cosmetids (Opiliones, Laniatores) yielded similar results, with the spider jumping on the harvestman, apparently biting its dorsum and letting it go unharmed (R. H. Willemart, personal observation). Although we cannot exclude the hypothesis that the cuticle itself is repellent to the spiders, we offer a plausible alternative explanation that does not require the involvement of repellent chemicals on the cuticle: a hard integument offers protection. Such a strategy has also evolved in vertebrates and other invertebrates. Pangolins, armadillos and millipedes are all known to protect their vulnerable ventral region by coiling up (e.g. Nowak 1999; Carrel & Britt 2009). Some beetles adhere themselves to the ground using adhesive foot pads to avoid being turned upside down (Eisner & Aneshansley 2000a). Some mites hide their legs and deflect the prodorsum (Sanders & Norton 2004), and gastropods hide inside their shell (e.g. Rochette et al. 2007). In contrast, the hard exoskeleton of *D. invalidus* also offers

protection on the ventral region and most parts of the legs (except for the tips of the legs, which bear sensory structures; Willemart et al. 2009), and the harvestman do not attempt to hide the few vulnerable parts from the spider. *Enoploctenus cyclothorax* did not search for such vulnerable parts when attacking the harvestmen (see Wigger et al. 2002; Řezáč et al. 2008).

The role of defensive secretions in this harvestman species remains to be investigated, but it might well be efficient against other predators, since distinct defensive mechanisms may be used according to the predator involved (Stuart-Fox et al. 2006; Botham et al. 2008; Schmitz 2008; Martin et al. 2010). Harvestmen may therefore be a 'beetle' among arachnids, relying on a heavy armature (see e.g. in beetles: Vander Meer & Wojcik 1982; Eisner & Aneshansley 2000a; Majerus et al. 2007) against predatory spiders. By ruling out a role for defensive secretions and demonstrating that such large predators can seldom pierce its integument, our study is the first to experimentally support the notion that these chemically defended prey are avoided by a predator because of their heavily built body and not because of their repellent chemicals. With empirical data showing its importance in protection against predators, one may see the harvestmans' heavily built body as a convergent solution similar to that of the elytra in beetles. Just as beetles are not very good at flying, species in the suborder Laniatores do not seem to be as fast moving as their counterparts in the suborder Eupnoi, which often have longer legs and do not have such heavily built bodies (although the integument may be hard in comparison with a spider, for example, see Fig. 5b). One may also wonder what are the costs associated with such a well-protected body and whether hard cuticles have evolved in less chemically protected species. We hope that all of these aspects will be investigated in the near future.

Acknowledgments

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