Acta Soc. Zool. Bohem. **80**: 39–44, 2016
ISSN 1211-376X

Conglobation as a defensive behaviour of pill millipedes (Diplopoda: Glomerida)

Ivan Hadrián Tuf[1)], Lucie Čmielová[1)] & Jan Šipoš[2)]

[1)] Department of Ecology and Environmental Sciences, Faculty of Science, Palacký University, Šlechtitelů 27, CZ–779 00 Olomouc, Czech Republic; e-mail: ivan.tuf@upol.cz
[2)] Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chitussiho 10, CZ–710 00 Slezská Ostrava, Czech Republic

Received 26 November 2014; *accepted 13 August 2015*
Published 12 April 2016

Abstract. Conglobation (rolling-up) is a typical defensive behaviour of pill millipedes (Diplopoda: Glomerida). Reactions of millipedes to a number of stimuli necessary to evoke conglobation and its persistence following three types of treatment were evaluated. The treatments were: touching, squeezing and dropping. Millipedes responded most strongly to being squeezed, but the longest duration of conglobation was recorded after repeated touching. In addition, to the response to the different types of treatment, the response and its persistence increased during an experiment, i.e. from the first to the third treatment.

Key words. Volvation, conglobation, death feigning, tonic immobility, Diplopoda, *Glomeris pustulata*.

INTRODUCTION

Volvation or conglobation is the adoption of a rolled-up posture, which is a typical defensive behaviour of several phylogenetically related groups of animals. Volvation occurs in mammals (armadillos, hedgehogs), mites (Oribatida), insects (cuckoo wasps), and crustaceans (Oniscidea). Among diplopods, volvation is typical of pill millipedes (Glomerida) and giant pill millipedes (Sphaerotheriida). This posture is basically defensive as the animal's legs and other delicate appendages are withdrawn inside the rolled-up body, which is protected by robust tergites and dorsal sheets and/or by spines as in extinct *Amynilyspes* Scudder, 1882 (Hannibal 1984) and hedgehogs. A thick cuticle is necessary not only for passive protection, but as support for strong muscles, which are necessary for maintaining this position in so called tonic immobility. This presents predators with a challenge; how to open the hard globe. Observations on mongooses in captivity and the field revealed that they can overcome this defensive mechanism by throwing millipedes at stones or trees (Eisner & David 1967, Eisner 1968).

The perfect enrolment of pill millipedes is possible because of particular adaptations: short body of subcylindrical form, emarginations in its terga (Hannibal & Feldmann 1981) and appropriate musculature. Associated with these adaptations is slow movement and inability to move laterally (Manton 1954). Upon encountering a predator prey individuals can run or feign death, but not both at the same time. King & Leaich (2006) report a negative relationship between tonic immobility and locomotor activity in a parasitoid wasp, which is similar to the slow movement exhibited by Glomerida that use volvation as their main defence (some Glomerida, but not Sphaerotheriida, also produce a repellent secretion; Shear et al. 2011).

Nevertheless, another possible function of rolling-up may be to minimise water loss by transpiration through the fine cuticle on the legs and ventral surfaces, which has been experimentally confirmed for pill bugs (Smigel & Gibbs 2008). The aim of this study was to evaluate role of conglobation in a pill millipede defence using different treatments.

MATERIALS AND METHODS

Pill millipedes *Glomeris pustulata* Fabricius, 1781 (Fig. 1a) were collected in April 2011, during an excursion to Hůrka u Hranic National Nature Reserve in Moravia, the Czech Republic. Local forests were damaged by a windstorm in summer 2008 and several gaps were created. Pill millipedes are very abundant under the bark of fallen beech trees in such

Fig. 1. The pill millipede, *Glomeris pustulata* Fabricius, 1781, is frequently found occurring at high densities under bark of fallen beeches: (above) pill millipedes eating faecal pellets of other phloem decomposers, (below) millipedes are abundant in their typical microhabitats.

gaps in forests (Fig. 1b). All millipedes were placed in a large plastic box with leaf litter and pieces of bark and kept at a constant temperature of 18 °C in constant darkness. One day before the start of the experiment, 150 millipedes (ca. 9 mm in size) were placed separately in small plastic boxes with moistened plaster of Paris at the bottom and leaf litter as food and shelter. All millipedes were tested five times on five different days over a period of three weeks; protocol followed previous research on terrestrial isopods (Tuf et al. 2015). Each experiment (one per day) consisted of three treatments, i.e. touching, squeezing and dropping in alternated order, with short breaks (ca. 30 min) between them. Touching was applied as a gentle nudge using the tips of pincers; this treatment was meant to resemble the touch of a small invertebrate predator. Squeezing was applied by holding the pill bug for up to 1 s using soft entomological pincers, which was meant to resemble being held by a bigger (vertebrate) predator. Dropping was applied by holding the pill bugs in pincers and dropping them from a height of 5–10 cm, which was meant to resemble manipulation by an even bigger predator, such as a bird or lizard. The order of these treatments was changed between experiments, i.e. each treatment was used as the first, the second or the third in the sequence, respectively. Each treatment was repeated up to five times and if volvation occurred its duration was measured up to the first sign of the animal resuming activity.

Data on the duration of volvation were analyzed using ANOVA tests and that on the number of stimuli using Pearson's χ^2 tests. Visualisations of results were done in Microsoft Excel programme.

RESULTS

A total of 150 pill millipedes were tested, 97% of which conglobated in reaction to the experimental treatments. We evaluated persistence, i.e. duration, of volvation following each type of treatment (touching, squeezing and dropping) and the sequence of treatment (the first, second or third), respectively (Fig. 2). The longest duration of volvation was recorded following touching (89 s), the shortest following squeezing (61 s) and the length of volvation was affected by the type of stimulus (Anova: F=6.00, p=0.003, Fig. 2a). The length of volvation was also affected by the order in which the stimuli were applied; there was an increase in the duration of volvation during the experiment from 62 s to 91 s following the third stimulus (Anova: F=6.24, p=0.002, Fig. 2b).

Another characteristic of the defence behaviour tested was the reactivity of pill millipedes to the order in which the different types of treatments were presented (Fig. 3). Reactivity can be measured in terms of the number of repeats of the same stimulus necessary to induce the millipede to adopt a rolled-up posture. There were significant differences in their reactivity to the different stimuli (χ^2=452.91, df=8, p<0.001, Fig. 3a), with rolling-up being induced by touching repeated 2.14 times and squeezing only 1.71 times. Also the order in which the stimuli were presented was important in determining millipede reactivity (χ^2=31.25, df=8, p<0.001, Fig. 3b), with the first stimulus having to be applied 2.10 times and the third stimulus only 1.85 times.

DISCUSSION

We evaluated the defence behaviour of pill millipedes using three types of stimuli and measured the duration of conglobation and number of repetition of each stimulus necessary to evoke volvation. These characteristics of its defence behaviour were associated with the type of treatment and order in which it was presented during the experiment.

Type of treatment

Reactivity and persistence of volvation was affected by the type of treatment. The longest response was evoked by touching; nevertheless a greater number of touching stimuli were needed to induce volvation. In contrast to their reactivity to touching, their reactivity to squeezing was more sensitive (a lower number of stimuli were required to induce conglobation), but the duration of conglobation was the shortest. This can be associated with the biological meaning of the different types of treatments. Touching is not a violent type of treatment and is similar to the random touching of another millipede when crowded. The pill millipede, *G. pustulata*, occurs under bark at high densities, so random touching of conspecific animals is probably a common event. To react to each random touching by volvation is unnecessary and time consuming. Nevertheless, if touched repeatedly at short intervals, it is similar to being manipulated by a small predator, such as a spider, ground beetle, ant or centipede (Quadros et al. 2012, Tuf et al. 2015). These small invertebrate predators repeatedly attack millipedes or wait until they un-roll; the pill millipede's longer tonic immobility in response to this stimulus is an adaptive response to this threat.

Unlike touching, squeezing is more similar to being manipulated by a larger vertebrate predator and therefore a fewer repeats of this treatment induces rolling-up. Small mammals, birds and lizards, can squeeze and loose their prey while manipulating it and are more likely to swallow the pill millipede whole than wait for it to un-roll. For this reason, it is not advantageous to remain rolled-up for a long time and therefore the duration of volvation was shortest following this stimulus. The association between squeezing and dropping is interesting. Volvation following dropping lasted for longer. If a big predator squeezes and then abandons a millipede for a while

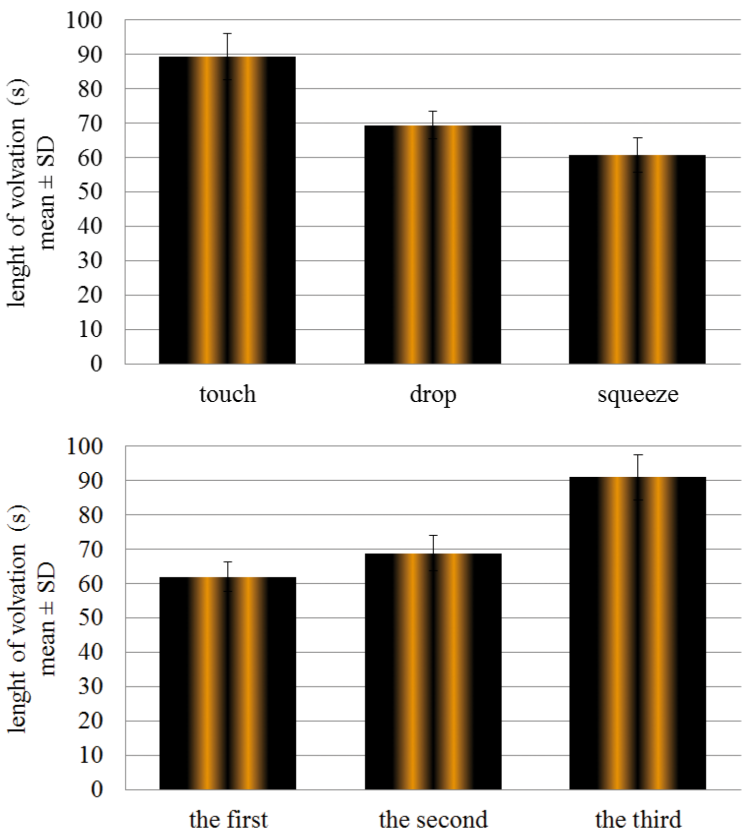

Fig. 2. Duration of the defensive posture of pill millipedes depending on (above) the type of treatment and (below) the order in which the different treatments were presented in each of the experiments.

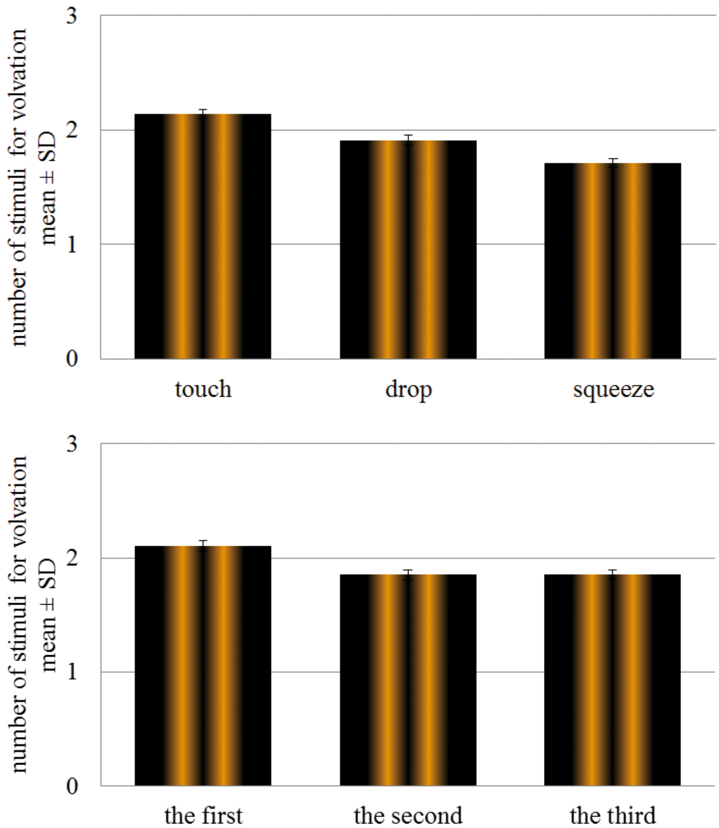

Fig. 3. Reactivity of pill millipedes depending on (a) the type of treatment and (b) the order the order in which the different treatments were presented in each of the experiments.

this possibly indicates that the predator has withdrawn. But if a predator squeezes a millipede and drops it from a height is likely the predator is searching for that millipede, so the longer it remains rolled-up the more likely the predator will give up searching for it.

Order in which the stimuli are presented

To evaluate the effect of the order in which the different treatments were presented, the order was different in the different experiments. In addition, to the type of treatment, it was evident, that both reactivity and persistence of volvation increased during an experiment. Millipedes subjected to repeated "attacks" conglobated more quickly and remained rolled-up for longer.

In addition, the reactivity of millipedes can be affected by its previous behaviour. Srinivasa & Mohanraju (2011) report that feeding millipedes are less likely to adopt a defensive posture than walking or resting millipedes. The greater incidence of pill millipedes responding to the second and third disturbance during the course of an experiment is probably due to their not feeding as a result of experiencing the first disturbance.

CONCLUSION

Pill millipedes conglobated readily in response to several types of stimuli, especially following squeezing (≈ attack by a large predator) and remained rolled-up for longer following touching (≈ manipulation by a small invertebrate predator). The highest reactivity and longest duration of volvation was induced by the third stimulus in the series in each experiment, irrespective what it was, i.e. millipedes became more sensitive to these stimuli during the course of an experiment.

Acknowledgements

This research was partly supported by Internal Grant Agency of Palacký University No. PrF_2014_021 and by European project No. CZ.3.22/1.2.00/12.03445, an Operational Programme for Cross Border Co-operation CZ-PL. The language of the manuscript was kindly checked by Professor Anthony F. G. Dixon (Norwich, UK).

REFERENCES

Eisner T. 1968: Mongoose and millipedes. *Science* **160**(3834): 1367.

Eisner T. & David J. A. 1967: Mongoose throwing and smashing millipedes. *Science* **155**(3762): 577–579.

Hannibal J. 1984: Pill millipedes from the Coal Age. *Field Museum of Natural History Bulletin* **55**(8): 12–16.

Hannibal J. T. & Feldmann R. M. 1981: Systematics and functional morphology of Oniscomorph millipedes (Arthropoda: Diplopoda) from the Carboniferous of North America. *Journal of Paleontology* **55**: 730–746.

King B. H. & Leaich H. R. 2006: Variation in propensity to exhibit thanatosis in Nasonia vitripennis (Hymenoptera: Pteromalidae). *Journal of Insect Behavior* **19**: 241–249.

Manton S. M. 1954: The evolution of Arthropodan locomotory mechanisms. Part 4: The structure, habits and evolution of the Diplopoda. *Journal of the Linnean Society* (*Zoology*) **42**: 299–368.

Quadros A. F., Bugs P. S. & Araujo P. B. 2012: Tonic immobility in terrestrial isopods: intraspecific and interspecific variability. *ZooKeys* **176**: 155–170.

Shear W. A., Jones T. H. & Wesener T. 2011: Glomerin and homoglomerin from the North American pill millipede Onomeris sinuata (Loomis, 1943) (Diplopoda, Pentazonia, Glomeridae). *International Journal of Myriapodology* **4**: 1–10.

Srinivasa Y. B. & Mohanraju J. 2011: To coil, or not to – activity associated ambiguity in defense responses of millipedes. *Journal of Insect Behavior* **24**: 488–496.

Smigel J. T. & Gibbs A. G. 2008: Conglobation in the pill bug, Armadillidium vulgare, as a water conservation mechanism. *Journal of Insect Science* **8**: 1–9.

Tuf I. H., Drábková L. & Šipoš J. 2015: Personality affects defensive behaviour of Porcellio scaber (Isopoda, Oniscidea). *ZooKeys* **515**: 159–171.