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Springtail Postmolt Vulnerability to Pseudoscorpion Predation: Mechanisms and Implications

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*Arthropod prey are expected to be more vulnerable to their predators immediately following molt. The effects of springtail (*Isotoma carpenteri*) postmolt vulnerability on interactions with a pseudoscorpion predator were examined in the laboratory. Springtails exposed to vials pretreated with pseudoscorpions (*Apochthonius minimus*) delayed molting compared to those prey that were exposed to vials pretreated only with springtails. Although their escape ability (measured as distance jumped) was unaffected by molt condition, postmolt springtails were more profitable in terms of reduced predator handling time following capture. Despite this,* A. minimus *did not distinguish between postmolt and intermolt prey presented at either end of a T-maze.*

KEY WORDS: *Isotoma carpenteri; Apochthonius minimus;* molt delay; escape ability; handling time; prey selection.

INTRODUCTION

Insects, and arthropods in general, must molt periodically in order to grow. Ecdysis, or molting, may result in loss of function of anatomical structures, causing an impairment of the senses, and of movement and defense, and thus result in increased vulnerability to predation (Chapman, 1982; Mill, 1985; Cromarty *et al.*, 1991; Steger and Caldwell, 1983). The predator of an organism that enters into regular periods of increased vulnerability could maximize its energy intake rate, and thus its fitness, by preferentially attacking the most profitable prey, i.e., those that are easier to capture, consume, or both. Preferential selection of recently molted prey has been reported in a variety of

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systems, including smallmouth bass versus crayfish, *Orconectes propinquus* (Stein, 1977), and stoneflies versus mayfly larvae, *Ephemerella subvaria* (Soluk, 1990). Thus, recent postmolt prey may face a dual disadvantage: an increased likelihood of attack and a decreased probability of escape in the event of an attack.

In the face of such a strong selection pressure, prey would benefit from the development of a behavioral mechanism to adjust molt timing to variation in predation pressure. Such a situation was suggested by Macchiusi and Baker (1992) in *Chironomus tentans* larvae. The presence of predatory sunfish caused a reduction in the proportion of third-instar midges that molted over a period of 7 days. Also, Harvey (1994) demonstrated that the xanthid crab, *Leptodius sanguineus*, delayed molting with increased risk of predation by *Thalamita crenata*, a portunid crab.

Springtails (Collembolans) are ubiquitous secondary decomposers, feeding upon leaf litter, feces and other organic debris. These small, soft-bodied insects are ametabolous, molting periodically throughout their life; *Isotoma carpenteri* Borner, the species used in this study, molts every few days (personal observation). For such an organism, the frequent periods of molt-associated vulnerability may have important consequences for an individual's survival. It follows that the timing of molting events may be of great importance to the springtail, even considering the insect's relatively soft intermolt exoskeleton.

Pseudoscorpions are small arachnids (<5 mm in length) that feed upon a variety of small prey and inhabit similar environments to springtails: humid soil, leaf litter, or otherwise sheltered environments. *Apochthonius minimus*, the predator used in our experiments, feeds primarily—perhaps exclusively—on springtails (Weygoldt, 1969; Johnson and Wellington, 1980). This species forages actively for prey after initial contact, in a manner similar to that described by Gilbert (1951) and Johnson and Wellington (1980). Prey are detected with the palpal setae, by touch or by sensing airborne vibrations. Approach is generally cautious, while attacks, effected by the pedipalps, are swift. The chelicerae receive prey immediately after capture. A prey item is ground up by the mouthparts and the resulting liquid–enzyme mixture is ingested; the remains are discarded in a small ball by wiping the chelicerae on the substrate.

The present study investigates several aspects of the pseudoscorpion–springtail predator–prey system. The objective of Experiment I was to determine if the handling times by pseudoscorpions of recently molted springtails (less than 24 h postmolt) are significantly shorter than the handling times of intermolt prey. Following this, and assuming that predation risk is temporally variable and springtails are able to detect predators or evidence of predation (perhaps through chemoreception), Experiment II tested the hypothesis that springtails delay their molt when the threat of predation is elevated. Experiment III investigated a possible mechanism of increased postmolt vulnerability: impaired escape ability. Specifically, we tested whether the jumping ability of springtails is compromised immediately after a molt. Pseudoscorpion prey choice was the subject of Experiment IV. If the handling times of molted prey are significantly shorter than those of intermolt prey (Experiment I), it would benefit pseudoscorpions to select recently molted prey over unmolted ones, to maximize their net energy intake rate. Thus, the objective of Experiment IV was to determine if pseudoscorpions select recently molted springtails in preference to intermolt ones.

MATERIALS AND METHODS

Rearing and Maintenance

Animals were collected in Confederation Park, Burnaby, B.C., Canada, in a humid area rich in leaf litter and well shaded by an extensive canopy. Litter samples were processed in a Berlese funnel and subjects aspirated from the collecting jar.

In the laboratory both predator and prey were housed in small (5.5-cm-high, 3.5-cm-diameter) plastic vials with an 8:1 plaster of Paris:charcoal base and perforated lids. The vials were kept at room temperature (varying from 20 to 28°C) in a covered bin with minimal light and high humidity, to approximate the natural conditions of moist humus. Humidity was kept high by moistening the plaster substrate of the animals' vials and maintaining a layer of water in the bottom of the storage bin.

Springtail groups (ranging from 1 to 40 per vial) were fed dried yeast pellets. Pseudoscorpions were individually housed to prevent cannibalism.

Experiment I: Springtail Handling Time

Each morning, previously isolated test springtails (0.45–0.55 mm in length) were checked for molts that had appeared overnight and their cells marked accordingly. A springtail was designated "molted" if it had produced exuviae within the past 20 to 24 h. The average molting rate for an individual springtail (determined by average group and individual rates) is approximately one molt every 5 days.

One or two trials were run each afternoon, according to scheduled predator feeding dates (pseudoscorpions were fed every 2 days) and availability of prey. Due to the unpredictability of springtail molting, molted subjects were capitalized upon when available. Unmolted prey were always in abundance.

The springtail, in its vial with lid removed, was placed into a carbon dioxide-filled chamber for a period of about 50 s. A small, premoistened brush was then used to transfer the anaesthestized insect into the center of the testing arena (the predator's vial, sheathed in paper towel to reduce light penetration) under a binocular microscope. At this time the springtail was measured from head to tail (furcula folded under abdomen) using an ocular micrometer. The center of the arena is least traveled by the predator, so placing the springtail there increased its undisturbed recovery time, ensuring that a trial did not begin before the prey left the center of its own accord.

Capture of the prey signaled the start of the trial and a stopwatch. After capturing a prey item, a pseudoscorpion may walk for a few seconds (up to a minute), apparently to find a suitable place to feed. The total handling time measurement (T_t) ended when the predator deposited the macerated prey item onto the substrate. Stationary handling time (T_s) was obtained by subtracting from the total handling time the time that a predator spent walking. The number of successful prey escapes (by jumping) was also noted.

Each of the 10 predators was retested 2 days later with a prey of different molt condition than it had encountered in the first trial. Despite this initial bias toward running trials using molted prey, the number of predators tested initially with unmolted springtails actually outnumbered those tested initially with molted ones (seven and three, respectively). Data were analyzed with a one-tailed, paired *t* test to test the hypothesis that 20- to 24-h postmolt prey require a shorter handling time than do intermolt springtails.

Experiment II: Group Molting Rate

In this experiment groups of five randomly selected springtails (0.40–0.60 mm long), all of which had been collected at least 2 days previously, to minimize stress and mortality, were placed into one of three vial types, differing only in their pretreatment. Vials designated Treatment 1 had contained a solitary pseudoscorpion, Treatment 2 vials had contained a pseudoscorpion and three springtails, and Treatment 3 vials had housed only three springtails. The first two treatments were to test the hypothesis that the prey react to the scent of predators and/or injured prey by delaying their molt. Treatment 3 acted as a control. For the sake of consistency, every vial contained a pellet of yeast even if it lacked springtails. Vials were established in groups of three, so that Treatments 1, 2, and 3 were set up simultaneously. Forty-eight hours after the pretreatment period began, all the animals were removed, along with any visible molts and eggs.

Five springtails were immediately placed into each of the three treatment vials using an aspirator. Any individuals that were stunned (i.e., motionless and unresponsive to gentle contact with a brush) were replaced. These groups were checked daily for egg and molt production for the next 4 days. Molts and eggs were counted (but not removed) and carefully mapped to avoid recounting errors.

Prey were not retested. In total, data were obtained from 102 groups of 5 springtails: 38 Treatment 2 vials, and 32 of both Treatment 1 and Treatment 3 (different sample sizes between treatments are due to springtail deaths; if more than two individuals had died before the end of the 4 days, that vial's data were rejected). Data were analyzed using a two-factor ANOVA (day, treatment) to test the hypothesis that springtails delay their molts when presented with evidence of predator presence and/or activity.

Experiment III: Postmolt Escape Ability

Isolated springtails (0.45–0.55 mm long) were checked each morning for the appearance of skin casts. When an individual was found to have molted it was randomly assigned one of four testing dates, 0.5 to 3.5 days postmolt.

On the afternoon of the assigned day the anaesthetized springtail (procedure as in Experiment I) was placed in the center of a flat plaster arena (12-cm diameter) above which was mounted a video camera. After a 2-min pause to allow for recovery, the subject was gently touched with a single hair brush on the rear of its abdomen, perpendicular to its long axis, producing a flight response (jumping and/or running). For each of 38 individuals, the distance jumped in response to the prod was recorded. A few individuals jumped twice; in such cases ($n = 3$) both distances were recorded. Data were analyzed by ANOVA.

Experiment IV: Prey Selection by Pseudoscorpions

The molting state of isolated springtails (0.45–0.55 mm long) was monitored daily as outlined above. On the day of a trial ($n = 10$) one recently molted (0.5 days postmolt) and one intermolt (>2 days postmolt) springtail were randomly placed in the arms of a small plexiglass T-maze. These side arms measured 1.9 × 0.7 cm, and the last 0.7 × 0.7-cm sections (containing the springtails) were cut off by Nitex screens (91 μm). The walls were 0.5 cm high, and the bottom of the maze (except for the springtail chambers) was covered with a 0.2-cm-thick layer of plaster. Approximately a minute after springtail placement, a predator (starved for a period of 2 days) was introduced at the far end of the long arm of the T (0.7 × 3.0 cm) and a clear cover placed over it; a 45-min overhead filming period then began. Time spent in either side arm of the maze was considered as selection of the prey item located there: once the pseudoscorpion's body was halfway into either region, timing began.

One-sample t tests (one-tailed) were applied to the arcsine square root transformed data, to test the hypothesis that recently molted prey are more attractive to pseudoscorpions than are intermolt springtails and that, as a consequence, pseudoscorpions will be more likely to spend time near them.

RESULTS AND DISCUSSION

Experiment I: Springtail Handling Time

The mean total handling time (T_t) for molted prey was significantly less than that for unmolted prey (Table I; $P = 0.041$). However, this statistic does not take into account the fact that pseudoscorpions frequently walk while holding their prey and may not be feeding at these times. Comparison of the total handling times minus predator time spent walking (i.e., the times spent stationary; T_s) yields a P value of 0.031: postmolt prey require nearly 3 min less handling time than do intermolt prey (Table I). Time spent walking (T_w) did not depend on springtail molt stage.

Which of the two variables is a more appropriate measure of handling time is not known for certain. The justification for the second statistic (with time spent walking removed) comes from observations of the feeding behavior of the predators. Once a prey item is caught it is alternately ground up between the chelae of the pseudoscorpion and ingested. Ingestion consists of the pseudoscorpion exuding enzymes onto the macerated prey item and subsequently reingesting the liquids. A mobile pseudoscorpion carrying a springtail in its mouthparts was never observed actively chewing; the prey were held motionless in the chelae. However, this does not exclude the possibility that the predator is still feeding in some way while walking—perhaps allowing its enzymes to digest its meal—and the time required for this may well depend on springtail molt state.

Unmolted and molted springtails were very similar in length (Table I), and variation in length of surviving test predators (many were lost to a microbial infection before data could be recorded) was less than 10%. Although there was some variation in predator starvation time before the trials, this had no apparent effect on handling time.

The average number of prey escapes during handling was actually greater for recently molted springtails than for unmolted ones (averages of 1.286 and 0.625 escapes per attack, respectively), but this difference was not significant (Wilcoxon $P = 0.59$).

Experiment II: Group Molting Rate

The molt time distribution data are summarized in Table II. There was a significant day effect ($P = 0.001$), and although there was no effect of treatment ($P = 0.58$), there was a significant day × treatment interaction term ($P = 0.048$). This indicates that the number of molts was the same in each treatment but that they were distributed differently in time: molting was delayed when the cells were pretreated with pseudoscorpions in Treatments 1 and 2 (Fig. 1).

Table I. Summary of Handling Times of Postmolt and Intermolt Springtails by Individual Pseudoscorpions[a]

Predator No.	Intermolt prey						Postmolt prey					
	Days starved	T_t (s)	T_w (s)	T_s (s)	Prey size (mm)	Escapes	Days starved	T_t (s)	T_w (s)	T_s (s)	Prey size (mm)	Escapes
1	2	590	63	527	0.5	—	2	710	70	640	0.5	—
2	1	1153	13	1140	0.53	1	2	396	20	376	0.45	0
3	1	649	0	649	0.55	0	2	190	0	190	0.45	—
4	1	478	0	478	0.52	0	2	351	127	224	0.55	—
5	2	664	0	664	0.5	—	1	525	0	525	0.56	3
6	3	669	22	647	0.55	0	2	643	0	643	0.55	0
7	1	309	60	249	0.55	0	3	339	133	206	0.53	1
8	4	376	0	376	0.5	0	2	438	0	438	0.5	0
9	2	666	0	666	0.55	4	4	444	11	433	0.52	5
10	2	363	70	293	0.55	0	2	238	0	238	0.5	0
Mean		591.7	22.8	568.9	0.530	0.625		427.4	36.1	391.3	0.511	1.286
SD		241.9	29.7	254.5	0.023			164.4	54.0	174.3	0.039	

[a] T_t is the total handling time, while T_w and T_s are the components of this time spent walking and stationary, respectively. Days starved refers to the predator starvation period prior to the trial. Escape of the springtail from the pseudoscorpion's grasp is also noted (a dash means that this information was not recorded by the observer).

Table II. Distribution of Molts Over the 4-Day Observation Periods in Experiment II

		Mean number molts/vial					
Pretreatment	*N*	Day 1	Day 2	Day 3	Day 4	Mortality (No./vial)	Eggs laid (No./vial)
1 (1 predator)	32	0.88	0.75	1.63	1.03	0.09	0.37
2 (predator + 3 prey)	38	0.53	0.63	1.03	0.79	0.29	0.29
3 (3 prey)	32	0.88	1.03	1.16	1.00	0.22	0.41

Even in Treatment 3 (control) fewer molts were produced on day 1 than on any other day of observation, indicating that handling alone causes a slight interruption in molting cycles. Harvey (1994) observed the same phenomenon in the crab *Leptodius sanguineus*, where daily handling was enough to cause an increase in the crabs' intermolt period compared to control individuals (no handling). In our case, it may also exaggerate the molting delays seen in Treatments 1 and 2, although the effect is much more pronounced in these treatments.

Despite the care taken to prevent harm to the springtails it was not uncommon for one or two (or infrequently, more) individuals to die during the 4-day experiment. This is of little concern because the bias it introduces is a conservative one. According to our hypothesis, Treatments 1 and 2 should cause a decrease in molting early in the 4-day test period, when the predator's scent is strongest. Any decrease in molts resulting from mortality late in the test period

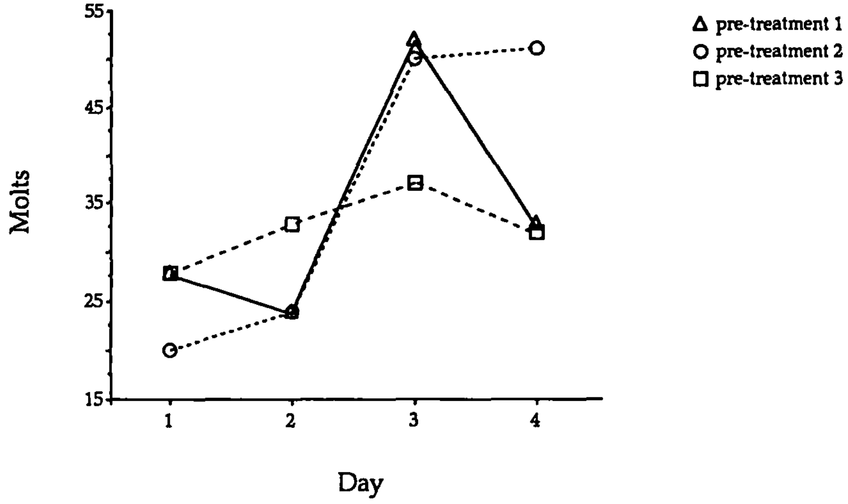

Fig. 1. Molt time distribution curves for springtails over a 4-day period in Experiment II.

would flatten out the molt distribution curve, reducing the treatment effect. Very early deaths (those on days 1 or 2) would have a neutral effect, decreasing the molting frequency both early and late in the test period. In any event, although there was a significant treatment effect on springtail survivorship (ANOVA; $P = 0.047$), the timing of mortality was independent of treatment (ANOVA; day × treatment interaction term, $P = 0.987$), so the results cannot be due to differential patterns of mortality. No significant trends in egg production were apparent.

Due to the large range in observed intermolt periods (from 2 to 9 days), it is not surprising that some springtails would molt twice, and others not at all, in the course of a 4-day experiment. In four cases in Treatments 2 and 3, and five cases in Treatment 1, six or more molts appeared over the 4-day period. A possible explanation for this is that some springtails were physiologically committed to the molting process at the time of collection, such that a delay in the cycle in response to a perceived increase in predation risk was impossible.

There are undoubtedly costs associated with delaying molting, namely, reduced growth and future reproduction. Springtails should therefore alter their molting behavior only when the benefit of doing so outweighs the costs. Presumably this will be true only if the predators detected do, in fact, discriminate between springtails in different stages of the molt cycle, and target recently molted individuals. This possibility is examined in Experiment IV.

Experiment III: Postmolt Escape Ability

Although jumping ability (distance) varied significantly with time postmolt (Table III; ANOVA; $P = 0.052$), there was no pattern apparent in these data; i.e., contrary to expectation, jumping ability did not improve as days postmolt increased from 0.5 to 3.5 days.

Interestingly, all three springtails who responded to brush contact by jumping twice were in the 1.5 days postmolt treatment group. The data were therefore reanalyzed using the total distance jumped by each springtail (in one or two

Table III. Response of Springtails to a Tactile Stimulus in Experiment III

Days postmolt	N	Distance jumped (cm) Mean	Distance jumped (cm) SD	No. of multiple jumps
0.5	15	1.771	0.659	0
1.5	15	1.250	0.701	3
2.5	9	1.591	0.396	0
3.5	7	1.924	0.380	0

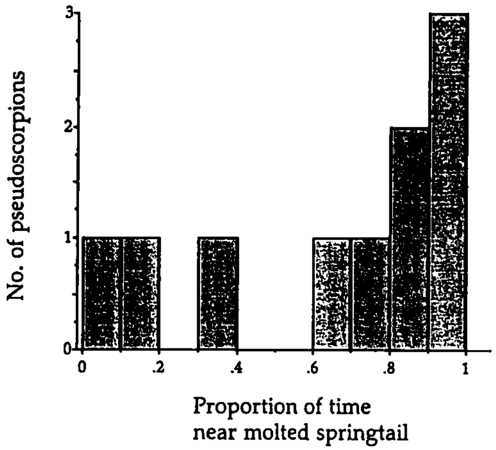

Fig. 2. Proportion of time spent by individual pseudoscorpions in the "molted side" of the T-maze in Experiment IV (as a fraction of the total time spent in either side arm of the T-maze).

jumps). The average total distance jumped on day 1.5 became 1.662 cm, and there was no significant effect of time postmolt on jumping ability ($P = 0.719$).

Experiment IV: Prey Selection by Pseudoscorpions

Although there was a tendency for individual pseudoscorpions to spend more time in the arm with the molted springtail (Fig. 2), this was not quite significant. A comparison of the arcsine square root transformed data to the random expectation (that 50% of the predator's time is spent in each end of the T-maze) yields a P value of 0.080 (one-tailed t test).

GENERAL DISCUSSION

Postmolt springtails appear to be more profitable food items than their intermolt counterparts, since the handling times of intermolt prey were significantly longer than those for postmolt prey (Experiment I). Pseudoscorpions should therefore prefer intermolt prey. This preference could be expressed in two ways: pseudoscorpions might be attracted to recently molted individuals, or they might reject intermolts.

Pseudoscorpions have primitive eyesight and hunt by touch using the setae on their pedipalps (which they sway from side to side while in motion). Prey are captured with the pedipalps and transferred to the chelae almost instantaneously. Rejection of a captured prey occurred in only one case: the springtail was discarded, only to be picked up and eaten approximately 10 min later. Pseudoscorpions are therefore unlikely to reject captured prey on the basis of molt condition. However, it is possible that chemoreception plays a role in prey selection by biasing encounters toward recently molted prey. For example, selection at the level of the food patch is possible, considering the tendency for springtails to molt at established areas or stations (personal observation).

Experiment II suggested that *Isotoma carpenteri* perceives *A. minimus* as a postmolt threat great enough to warrant a delay of their molt. If *A. minimus* does not preferentially select recently molted prey, springtails would not benefit by adjusting their molting behavior in the way we observed—unless postmolt springtails are more vulnerable to capture. Experiment III provides no evidence for this: jumping ability did not appear to be affected by molt condition.

Thus, the springtails' response to predator odor leads us to the conclusion that the predator must preferentially attack recently molted springtails, despite the fact that the results of Experiment IV do not support this. Since there was in fact a nonsignificant tendency for pseudoscorpions to select recently molted prey in that experiment, a larger sample size (both prey and predators), or stronger chemosensory stimuli, might produce more conclusive results.

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