The injection of venom by a spider
(Cupiennius salei, Ctenidae) and the weight of an insect prey (Acheta domesticus, Gryllidae)

par
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Summary: A series of prey items of a given weight were offered to a spider. They were obligatory removed 1 min after the beginning of the attack, and their state noted afterwards. Venom is injected in small as well as in large prey. The prey had 37 to 520 % of the spider weight. The mortality rate of the prey is negatively and linearly correlated with their weight. When a prey weighs about 30 mg or more, the spider (15 mg) injects almost all its venom in the first one of the successive prey items. With smaller prey, the venom glands are gradually emptied during the successive bites.

1. INTRODUCTION

The starvation level of a spider, the relative sizes of spider and prey, and the palatability of the prey are important parameters determining if a spider will attack a prey and if the spider will be successful (NAKAMURA 1987; NENTWIG 1987). Even when a spider subdues a prey, we do not know how much venom is injected and which conditions influence the injection. It may be of prime importance for the spider to modulate, for example, quantity of venom injected and injection rapidity, in function of eco-ethological parameters. In this paper, I answer one main question. Which influence has the prey size on the amount of venom injected?
2. MATERIAL AND METHODS

2.1 Spiders
From a laboratory rearing, neonate spiders of *Cupiennius salei* Keyserling were kept individually in polystyrene boxes (Ø5 X 8 cm) closed by a foam-rubber plug. A bottom of plaster, 2 cm thick, was humidified weekly. The spiders were fed weekly with about ten young *Acheta domestica* (individual weight of about 1 mg). This cricket species was also used in the bio-assays.

All spiders used in the bio-assays were of instar IV (prosoma length: 2.2-2.5 mm) which lasts 3-4 weeks. When a spider was of this instar for at most one week, it was starved for 2 weeks. It was then used in a bio-assay only when weighing 10.0-20.0 mg.

2.2 Bio-assay
A prey of known weight was placed in the polystyrene box and remained there for a maximum of 10 min. When the prey was caught by the spider, I removed the prey exactly 1 min after the beginning of the attack. The prey was removed by handling the spider with forceps until it released it, which took not more than 10 sec. A second prey item was placed in the box of the spider, 10 min after the deposit of the first one. When this one was also caught, it was removed in the same way, and so on. A maximum of 12 prey items were successively offered per spider, except for prey of more than 30.0 mg (see below); from them a maximum of 3 individuals were offered. When a prey was not attacked during the 10 min, the bio-assay was finished for the spider which was never used again.

Prey of the following weight categories were used: 5.0-5.9, 7.0-7.9, 9.0-9.9, 11.0-11.9, 13.0-13.9, 15.0-15.9, 17.0-17.9, 19.0-19.9, 23.0-24.9, 29.0-30.9, 35.0-36.9, 41.0-42.9, 47.0-48.9, 58.0-61.9, and 76.0-79.9 mg. Throughout the text, each category will be designated by its central value. Each spider received prey items of one category. The number of spiders which attacked at least one prey was 24 for those receiving 5.5 and 7.5 mg prey items, and 12 for all others.

In order to compare the effect of the venom with the mechanical effect of the bite onto the prey, the venom glands of spiders were emptied a day before the experiment. By electrical stimulations (9 V) onto the chelicerae, venom droplets appeared, and were removed with filter paper. 12 spiders were tested with 7.5 mg prey items and 12 spiders with 15.5 mg prey items (maximum: 3 per spider).

Venom could be injected during the removal of a prey from the spider. The influence of this possible artifact was assessed. After a 1 min bite, spider and prey were placed during 5 sec under vapors of ether. The prey was then easily removed since the spider was partially anesthetized. This method was not used more generally in the bio-assays, the repeated use of the solvent being toxic for the spider. Prey kept for 5 sec under the vapors were intoxicated only for a short time. 12 spiders were tested with 15.5 mg prey items.
2.3 States of the prey
Each bitten prey item was kept in a humid Petri dish. One hour and 24 h after the experiment, it was gently handled with forceps: it was possibly placed on its back, thorax and abdomen were touched, the cerci pinched. The prey, observed under the microscope, was then in one of the following seven states: "normal" (i.e. normal reactions), "normal-abnormal" (walk normally but rarely jump in order to run away), "abnormal" (walk abnormally), "abnormal-immobilized" (able to turn over with great difficulty when placed on its back), "immobilized" (not able to turn over, but carry out rapid movements of at least one part of the body), "immobilized-dead" (carry out very slow movements of one part of the body), "dead" (absolutely motionless).

3. RESULTS AND DISCUSSION

3.1 State of the prey 1 h and 24 h after the bite of a spider
Almost all 5.5 mg prey items are dead 24 h after the experiment. Most prey items weighing at least 7.5 mg, and which are "abnormal-immobilized" to "normal" 1 h after the experiment, are in a same or a better state 24 h after the experiment (figure 1).

Fig. 1. - Distribution of the prey in function of their state 1h (Z axis) and 24h (X axis) after the bite of a spider. The states of the prey (n) "normal", (ab) "abnormal", (i) "immobilized", (d) "dead", and the 3 intermediate ones are described in the text (see 2.3.). Prey weight categories of (a) 7.5 mg, (b) 15.5 mg, and (c) 30.0 mg (n=116, 65, and 35 respectively).
Thus these prey are able to recover. In contrast, most prey first “immobilized” are later either in the same state, or “abnormal” to “normal”, or “dead”. Finally, almost all “immobilized-dead” individuals die afterwards. Thus, it seems likely to consider three groups: “abnormal-immobilized” to “normal”, “immobilized”, and “immobilized-dead”+“dead” prey. Throughout the text, these categories will be designated by normal, immobilized, and dead, respectively, and will be given for the results 24 h after the experiment. Prey weighing at least 30.0 mg are either normal or immobilized+dead (figure 1c). Thus, the prey state is generally not highly different between 1 h and 24 h after the spider bite. Such a temporal stability is also found in several insect species injected with pure venom of C. salei (FRIEDEL & NENTWIG 1989).

3.2 The effect of the venom and the mechanical effect of the bite
The number of prey alive (i.e. being immobilized or normal) is high when they were attacked by a spider with emptied venom glands (8 of the 12 prey items weighing 7.5 mg survived, 11 of those weighing 15.5 mg; first prey attacked by the spider) and is significantly lower when attacked by a spider with venom (P<0.001 each time for both weight categories, Fisher exact probability test; see also table 1). For the second and third prey attacked, significant differences are achieved with 7.5 mg prey (P<0.05 two times), but not with 15.5 mg prey (P>0.05 two times, considering the relatively high survival rate, even when the prey are bitten by spiders with venom). These results suggest that in the bio-assays, mortality is principally due to the venom, and not to the mechanical effect of the bite.

The 15.5 mg prey items removed under ether vapors, in comparison to those removed with forceps, have a similar number of dead and of immobilized+normal prey (P>0.05 three times, i.e. with the first, second, and third prey attacked, Fisher exact probability test). Thus, the spider does not seem to inject venom when handled with forceps.

3.3 State of the successively attacked prey and prey size
The first attacked prey items (see p1 in table 1) are more frequently dead than alive (immobilized+normal) when they weigh 7.5 mg. When the first attacked prey is compared to the further ones, it is not before the seventh prey item (p7) that the numbers of dead and of alive individuals are significantly different (P<0.05, Fisher exact probability test). Differences are always significant (P<0.05; table 1) when the first prey item is compared with the fifth one (weight categories of 9.5 and 13.5 mg), the fourth one (15.5 mg), and the third or second one (17.5 to 42.0 mg). The higher mortality rates of the first prey may be explained in two ways. 1) The venom glands are gradually emptied during the successive bites and the last prey items receive no venom. 2) Venom glands are never totally emptied during the bio-assay, because the spider “learns” to inject in the last prey items a smaller amount of venom (i.e. far under the lethal dose). The latter hypothesis could be valid for small prey (e.g. of 7.5 mg) which are relatively easily handled by the spider. In contrast, large prey escaped sometimes from the spider (namely 1, 2, 2, and 5 prey items in the 36.0, 48.0, 60.0, and 78.0 mg categories, respectively; first prey attacked).
Table 1. - Number of prey items which are (n) normal, (i) immobilized, or (d) dead, 24h after the bite of a spider. The spiders received successively the prey items (p1) to (p12). Prey weight categories from (5.5 mg) to (78.0 mg).
Thus, the "raison d'être" of the venom is especially obvious when a spider subdues a prey which is large and dangerous (e.g. by kicks of the posterior legs).

Fig. 2. - Number of dead prey, 24h after the bite of a spider, in function of the prey weight. First attacked prey by the spider. Data refer to table 1.

Mortality (M) of the first attacked prey is linearly correlated with (W), the prey weight (M=-1.27W+103, r= -0.93, P<0.001, n=15; figure 2). This suggests that mortality could depends directly on the sensitivity of the prey to one given amount of venom. A spider seems to inject this given amount of venom, i.e. all its venom, in one prey which weighs more than 15.5 mg. The more the prey then increases in size, the more it survives the bite (see figure 2) since it received, proportionally to its weight, less venom.

Thus, the venom glands are emptied, even by giving the spider a series of small (about 7.5 mg) prey. In natural conditions, at least a small amount of venom is probably injected in prey of any size. On the other hand, the spider does not seem to learn, during my experiments, to economize venom when handling one after another relatively undangerous prey items.

3.4 Number of successively attacked prey and prey size
The mean number of prey attacked per spider ranges from 3 to 6 (standard deviation: 2 to 4) in each one of the prey weight categories from 5.5 to 30.0 mg. Very large prey are rarely attacked. The number of spiders, tested in order to obtain 12 attacking at least one prey, was 39 with 78.0 mg prey items, when 12-19 with 9.5-60.0 mg ones. Moreover, large prey are attacked in small numbers (table 1). But the reverse is not necessarily true. For instance, only a few spiders attacked 12 prey items of 7.5 mg
There is a rather constant decrease in the number of spiders which attack an increasing number of prey items. Thus, when a prey was taken, the probability that a spider will take a next one remains fairly constant at any moment of the bio-assay. This could be due to the loss of energy, consumed for subdueing each prey, and to a behavioural fatigue. In contrast, the availability of venom in the glands does not seem to influence the probability of an attack. Even with glands emptied by successive bites, or by removing the venom (see 3.2.), a spider can attack several prey items.

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