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SHORT COMMUNICATION

Rabidosa rabida (Walckenaer, 1837) (Araneae: Lycosidae) does not require venom injection to capture prey in the lab

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Abstract. Spider venom is assumed to be used primarily to subdue larger prey and secondarily in defense. *Rabidosa rabida* (Walckenaer, 1837) is a non-web building, venomous spider. Its feeding behaviors suggest venom may not be as important as previously expected in prey capture and immobilization. We conducted feeding tests to examine the importance of venom injection in prey capture for *R. rabida*. Groups of large crickets were offered to two groups of adult female spiders with either functional or glue blocked venom pores but otherwise functional chelicerae. Our results could not confirm a significant effect of venom availability on prey capture and showed that spiders could immobilize prey without the use of their venom. These results expand upon previous studies suggesting prey capture was possible without the use of the fangs, but prey immobilization required venom. This study suggests our understanding of spider prey capture and venom use is incomplete.

Keywords: Chelicerae, fang, prey immobilization

Spiders are the most abundant terrestrial predators and are ecologically important because of the large number of arthropods they consume (Uetz 1992; Foelix 2011; Nyffeler & Birkhofer 2017). Studies of spider predation have mainly focused on the use of webs and venom to immobilize prey and avoid predator injury. For non-web building spiders, venom has been the primary focus of studies on the mechanics of predation. Venom can be defined as a glandular secretion containing molecules that disrupt normal physiological processes, that is delivered from the animal that produces the secretion into a target animal in order to facilitate feeding, defense, escape, or some other fitness-improving practice of the producer (Casewell et al. 2013). Spiders secrete their protein-rich and metabolically costly venoms from glands located within the prosoma. This fluid is then delivered from the gland into the target through the fang. The fangs possess a small, sub-terminal venom pore through which venom is expressed by muscular contraction of the venom glands and ducts. In addition to the venom pore, the fang also possesses cuticular features such as tooth-like projections or serrations and muscles which allow the chelicerae to be used mechanically for manipulation of web and/or prey (Foelix 2011). Other physical factors involved in prey capture, such as adhesive hairs, have been described for spiders (Rovner & Knost 1974; Rovner 1978), but it is still widely assumed that venom is the most important factor affecting any venomous spider's ability to capture prey (Foelix 2011).

Rabidosa rabida (Walckenaer, 1837) is found across the eastern half of North America (Brady & McKinley 1994) and occurs in high density and abundances. This spider has a large adult body size and is capable of capturing prey slightly larger than itself (Stork 2011). Indirect benefits for the plants where it hunts nocturnally, such as reduced plant damage due to herbivory, have also been shown for this spider (Schmitz & Suttle 2001). Observations of *R. rabida*'s hunting and prey capture behavior as well as a commonly cited paper (Rovner 1980) initiated interest in the use of venom for prey capture for *R. rabida*. Rovner (1980) examined physical factors involved in prey capture by *R. rabida*, including scopula hairs on the legs, musculature and tooth-like ridges on the chelicera, and use of the basal portion of the chelicera. He suggested that this species is able to use these morphological features to grasp a cricket, but is unable to immobilize prey without the use of the venom from its fangs. The methods used by Rovner (1980) raise the question of whether it was the lack of venom injection that caused the inability to immobilize prey. In that study, the entire distal portion of the chelicera was sealed into the cheliceral grooves with wax. This resulted in both venom and the ability to naturally manipulate prey with the chelicera being unavailable. In the lab, when *R. rabida* comes into contact with multiple prey items, it will often smash these prey items together into an amorphous mass before consuming them. The observed physical manipulation of prey suggests a reduced need for venom. However, *R. rabida* hunts up in the vegetation, often without a place to chase prey, and this could suggest an increased need for venom (Binford 2001). The speed of the crushing behavior observed in spiders from Arkansas appears to support a prey capture method that would not rely on venom. We tested whether *R. rabida* would be able to capture and consume prey with and without the ability to express venom from its venom pores. We hypothesized that the ability to inject venom would not affect the proportion of prey captured and immobilized by these spiders in the lab.

To test if venom was necessary for all prey capture behaviors, we captured adult female *R. rabida* from a field adjacent to a small body of water just off the public bike trail in Searcy, Arkansas (35.26°N, 91.72°W). The spiders were housed in the lab at Harding University where they were kept in 16 × 14 x 7.5 cm clear plastic boxes on a 14:10 L:D light cycle at 25°C and were provided water *ad libitum* via cotton-stopped shell vials. Once acclimated to the lab for a week, spiders were offered 10 large crickets for 24 hours to standardize their hunger. All live crickets and cricket remains were removed from the boxes after 24 hours. All spiders that ate either 0 or 10 crickets were removed from the test along with any spiders that molted, laid an egg sack, or showed any reduction in coordination or ability to move around its enclosure. Following hunger standardization, we measured the spiders' carapace length, carapace width and mass. The spiders were divided into two groups by ordering the body size from largest carapace length to smallest. We then placed every other spider into the first group and the rest into the second group so that body sizes were distributed equally. In one group, we placed super glue (ethyl cyanoacrylate, Krazy Glue©) over the venom pore of their fangs, filling the pore and blocking the flow of any fluid through the opening. To glue a spider, we placed it into a transparent, plastic sandwich bag and pulled it tight, so that the spider was restrained but

Table 1.—Results of ANOVAs comparing size corrected proportion of prey captured by spiders with their venom pores glued shut and unglued groups of spiders. See text for description of tests 1 and 2.

Source of Variation	Test #1	$n =$ 126 spiders			Test #2	$n =$ 197 spiders		
	df	MS	F	*P*	df	MS	F	*P*
Group	1	0.003	1.406	0.238	1	0.003	1.137	0.288
Error	124	0.002			195	0.002		
Power estimate	0.211				0.157			

unharmed. We placed the spider on its back to expose the ventrally pointing chelicerae, and then cut a small hole in the bag just over the chelicerae. A small amount of super glue was placed in a 0.8–1.0 mm capillary tube, which fit snugly over the distal tip of the fang and immersed the fang pore in the glue. When the tube was removed, glue remained, filling the venom pore. After allowing the glue to dry, we observed each fang under a dissecting microscope to ensure the glue filled the venom pore and blocked it completely.

Spiders in the unglued group were restrained the same as the glued group and an empty capillary tube was placed over each fang. Both groups then had the effectiveness of the gluing tested using electrostimulation with a TENS pain relief kit (Medical Products Online Inc.). This battery-operated system provided a burst charge with a pulse width of 80 μS and a pulse rate of 120 Hz. Bare lead wires and a drop of tap water were used to allow the point of stimulation to be applied to the sides of the prosoma close to the venom glands of the restrained spider. All spiders recovered quickly and completely from the shocks. Any spider that expressed fluid from the blocked pores was removed from the experiment or re-glued. Following the feeding trial, electrostimulation was again used to check that the pore blockage was not dislodged during feeding and any spiders that expressed venom were removed from analysis. The control group was handled in the same way as the glued group with the exception of the glue application to control for the potential effects of handling on feeding behavior.

Following the gluing procedure, spiders were starved for two weeks to allow for appropriate hunger and venom production. Following two weeks of starvation, the spiders were offered 20 large crickets for 24 hours. Crickets were 20 mm in body length, just under the mean body length of 20.7 mm (SD ± 1.6) for the spiders we collected during our first test. The proportion of crickets captured, and at least partially consumed, was recorded for each spider. The proportion of prey captured was calculated by dividing the number of killed and partially consumed crickets by the number offered. To scale for spider size, we divided the proportion of prey captured by carapace length. We used carapace length instead of the carapace width, because it allowed us to meet parametric assumptions in this test and has been shown in past work using *R. rabida* from Arkansas to have a significant effect more often than carapace width (Stork 2011). The proportion of prey captured controlled for body size was square-root transformed to meet parametric assumptions. We compared the proportion of prey captured, controlled for body size, between the glued and unglued groups using analysis of variance ($n = 56$ glued and 70 unglued spiders). A power analysis was run in SYSTAT 11.0 (2004) using the smallest group's sample size.

We ran a second feeding test to determine if venom loss during electrostimulation before the feeding test in the unglued group would change the results. No spiders were used in multiple tests. Spiders for the second test were captured a month later in the summer using the same methods and capture location as in the first test. In the second test, the methods were the same as described above except that electrostimulation was conducted on both groups a week after the feeding trial rather than both before and after. We did this so that the unglued group, which would have lost venom during testing, would not have to regenerate its venom during the period of starvation before the feeding test. If venom regeneration were incomplete, it may have put this group at a prey capture disadvantage and potentially reduced the appearance of any differences between the groups. In both tests, we observed glued fangs under a dissecting microscope before the feeding test, to ensure that the fang pores were completely filled with glue. Another difference in methods in the second test was that the crickets used in the second test were slightly smaller than those in the first test (body length 15–20 mm). Given that spiders in the second test were older and thus larger than spiders in the first test (21.6 mm ± SD 2.2), these crickets were relatively smaller. During the second feeding test, 197 spiders were tested. Glued spiders that expressed venom at electrostimulation following the feeding test were removed from the feeding test, leaving 73 spiders that did not express venom. We also tested 124 unglued spiders. The data were analyzed as in the first test. We also made qualitative observations of prey capture behavior.

Spiders that were not able to inject venom showed no significant difference in the proportion of prey captured and killed, corrected for body size, compared to the spiders that were able to inject venom. This was consistent in the first ($F = 1.406$, $P > 0.23$, $df = 1$) and second ($F = 1.137$, $P > 0.29$, $df = 1$) runs of the test (Table 1; Fig. 1). The power (the likelihood of correctly detecting a difference between the groups) for the first ANOVA was 0.211 for $df = 1$ and $n = 56$. The power of the second ANOVA was 0.157 for $df = 1$ and $n = 73$ (Table 1). To achieve a power of 80%, a sample size of 325 and 623 spiders in each group would be required for the first and second tests respectively.

Observation of prey capture behavior, such as the ability to grab and subdue multiple crickets at one time, did not suggest any difference between spider groups. All spiders were able to grab and subdue prey without any obvious difficulty. Most spiders from each group were able to capture multiple crickets at one time.

Our results show that, in at least some prey capture situations, venom is not vital to *R. rabida* for subduing prey, contrary to what was previously assumed. Our power was very low due to there being almost no difference between the means of each group and large variation in the proportion of prey captured for both groups. This means that a difference could exist that we were unable to show in these tests because our sample sizes were not over 600 spiders. It is possible that venom aids in prey capture, though we were unable to show that here, but it is not necessary for prey capture as even spiders that were unable to express venom were able to capture prey with no apparent difficulty. These results suggest that physical manipulation may be more important than venom in prey capture for *R. rabida*.

Our results contrast with those of Rovner (1980), who found that spiders were unable to immobilize the cricket they captured when the fang was immobilized and venom was not able to be used. We suggest that the inability to subdue prey in Rovner's paper was likely due to the inability to use the entire chelicera, though we did not directly test that here. More work needs to be done on this system to examine previous assumptions and to see if larger, more difficult, or more dangerous prey might require venom for capture by the generalist *R. rabida*.

Rovner (1980) also addressed the question of whether venom allowed for predation on larger prey. Because the spiders were unable to immobilize prey without the fangs, he concluded that venom allowed for capture and ingestion of larger prey (Rovner 1980). This conclusion is called into question by our results, as venom was not necessary to capture and consume prey if the fang was mobile and able to be used in prey immobilization. More recent studies of venom and prey size have shown a link between amount of venom used and

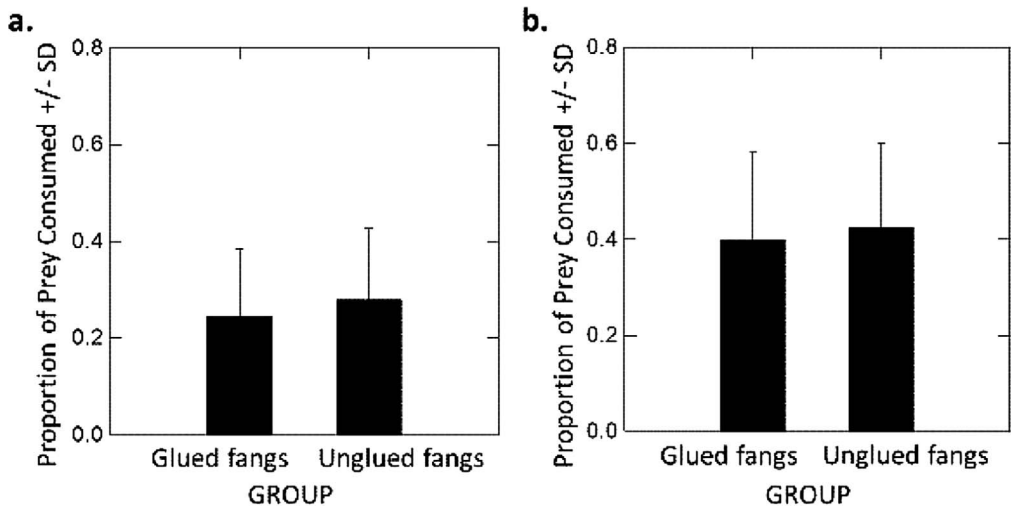

Figure 1.—A comparison of the mean proportion of prey captured and consumed by spiders with and without venom use. (a) First test conducted in early summer. (b) Second test conducted in late summer and with smaller prey size relative to larger spider size.

prey size in *Cupiennius salei* (Keyserling, 1877) (Malli et al. 1998). Other tests have shown that prey size in conjunction with the ease of handling have been found to be more important than size alone (Malli et al. 1999). These studies have advanced our understanding of the role of venom in predation by wandering spiders, but have focused almost entirely on the ctenid *C. salei*, which is found in Eastern Mexico, Guatemala, Belize, and Honduras. These questions have not been applied adequately to other spiders, including those in North America. We would like to see further exploration into the characters affecting prey capture and the role of venom and digestive fluid for *R. rabida* and other families of wandering spiders. A better understanding of the role of venom in prey capture for spiders in general is dependent on diversifying the species used to address these questions and looking at how consistent the dependence on venom is between families (Rovner 1980).

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