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Temporal variation in venom yield of the Australian funnel-web spider *Atrax sutherlandi* (Hexathelidae: Atracinae)

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Summary

Temporal variation in the venom yield of spiders is a relatively poorly understood phenomenon. We investigated temporal variation in venom yield of the Australian funnel-web spider *Atrax sutherlandi* Gray 2010 (Hexathelidae: Atracinae). The venom yield of spiders collected and milked in winter was 62.9% higher than those collected and milked in autumn, despite all undergoing acclimatization (45 days in darkness at 10°C and 100%RH) before milking. Our findings highlight the potential effects of seasonality on spider venoms and lay the groundwork for future studies to investigate the evolutionary and ecological correlates of this phenomenon further.

Introduction

Intraspecific variation in the quantity of arachnid venoms (i.e. venom yield) may be associated with ontogenic, sexual, diet and temporal factors (de Oliveira *et al*. 1999; Herzig, Ward & dos Santos 2004; Herzig 2010). Of these, data on temporal variation in venom yield is the least well documented, and generally based on anecdotal evidence from opportunistic observation (e.g. Bücherl 1953; Wiener 1956; Wiener 1959; Schenberg & Pereira-Lima 1966) with little discussion of underlying ecological and biological mechanisms.

The neurotoxic venoms of the Australian funnel-web spiders (Hexathelidae: Atracinae) have been subject to extensive toxicological research, with the majority of work focusing on the notorious Sydney funnel-web *Atrax robustus*. The venom yield of this species has been observed to vary significantly by season, with the highest yield reported in spring (Wiener 1959). The present study focused on a recently described species of the genus, *Atrax sutherlandi* Gray, 2010, which is distributed across temperate forests in most of south-eastern New South Wales. This fossorial spider constructs permanent silk-lined burrows (*c.* 10–30 cm deep) in the soil beneath fallen logs and rocks. Females appear rarely to leave the immediate vicinity of their burrows (pers. obs.), in contrast to the vagrant males, which have been collected from pitfall traps and temporary refuges built under rocks in summer months (unpublished data). During winter, the spiders appear largely inactive and retreat deeper into their burrows (pers. obs.). In this study, we investigate temporal variation in venom yield of *A. sutherlandi* by comparing the venom yield of spiders collected and milked in two different seasons.

Methods

In 2014, adult females were collected from Tallaganda forest (35°15′S–36°15′S 149°28′E–149°37′E) in two separate batches: 23 specimens on 2 April (mid-autumn) and 22 specimens on 2 July (mid-winter). Only large spiders from well-established burrows were collected to avoid including pre-reproductive males (which cannot be distinguished from females on external morphology alone). Following collection, the spiders were housed individually in 70 ml plastic specimen jars at 10°C and 100% RH in constant darkness for 45 days, in preparation for venom milking. The dark setup was intended to approximate light availability within a burrow in the field, and also acclimatize all spiders to the same conditions. Spiders were fed successfully once every three days with a medium-sized (~15 mm) *Tenebrio molitor* larva for the first 39 days, but not fed in the last six, to allow replenishment of venom reserves in the absence of prey capture prior to milking. No individuals moulted while in captivity.

After the 45-day acclimatization period, venom was milked from each specimen in a 5-minute sitting daily (between 0900 and 1600 hrs) over three consecutive days (autumn batch milked 17–19 May; winter batch milked 16–18 August). Pilot tests showed this regimen depleted the venom stores of 97% of subjects. During each sitting, the spider was repeatedly provoked by touching its front legs with metal forceps, causing it to produce venom on the tips of its fangs, which was milked via capillary action using a 32 mm (5 μL) microcapillary tube (Model: P1799, Drummond Scientific, USA). The tubes were photographed with a digital microscope (Model: P-400Rv, Nikon, Japan) immediately after every sitting, and the length (mm) of each tube occupied by venom was measured and converted to volume (μL) via image analysis using the software ImageJ (Abràmoff, Magalhães & Ram 2004). The absolute yield (Y_a) of each spider was the total venom quantity collected over the three-day period.

Statistical analyses were performed using SPSS 22.0 (IBM Corporation 2013). To derive a size-corrected measure of yield, carapace width (CW) of each specimen was measured as an indicator of body size (Hagstrum 1971), then Y_a was regressed against CW, CW^2 and CW^3 and the resultant coefficients of determination (r^2) were scrutinized to determine the dimension of body size (i.e. width, surface area or volume) that was most strongly associated with yield. This measurement was then utilized for subsequent correction of absolute yield for body size, and comparison of size-corrected yield between the autumn and winter batches.

Results

Among CW, CW^2 and CW^3, CW^3 showed the strongest relationship with absolute yield (Y_a) ($r^2 = 0.407$, $p < 0.0001$) (Fig. 1). Size-corrected yield (Y_s) was calculated using the equation: Y_s (μL mm^{-3}) = Y_a (μL) / CW^3 (mm^3)

There was no significant difference (p = 0.634) in the mean size (CW) of spiders between the batches from autumn (6.76 ± 0.94 mm) and winter (6.71 ± 1.00 mm). However, there was a significant difference in the mean size-corrected yield (Y_s) of autumn (0.0447 ± 0.023 μL mm^{-3}) and winter (0.0728 ± 0.0259 μL mm^{-3}) batches, t (47) = -3.44, p = 0.001, d = -1.15 (Fig. 2).

Discussion

In our assessment of the relationship between venom yield and body size in *A. sutherlandi*, we found absolute yield to be most strongly associated with the volumetric dimension of body size (indicated by CW^3). This may be explained by the changing volume of the venom glands with overall body size influencing venom production and venom-holding capacity (Herzig, Ward & dos Santos 2004). Similar relationships have been demonstrated in other spiders including *A. robustus* (e.g. Wiener 1959; Vapenik & Nentwig 2000). The linearity of the relationship between venom yield and overall body size (Fig. 1) is consistent with results from other mygalomorphs (Herzig 2010) but contrasts with exponential modelling reported for araneomorphs (e.g. Malli, Vapeli & Nentwig 1993; Herzig, Ward & dos Santos 2004). Herzig (2010) attributed such disparity in size-yield relationships between the infraorders to differences in (i) venom gland morphology, whereby mygalomorph venom glands are restricted to the basal part of the chelicerae compared to araneomorph venom glands that extend into the prosoma (Foelix 2011), and (ii) moulting, whereby unlike adult mygalomorphs that may increase venom yield with moulting and growth, araneomorphs generally do not moult after reaching adulthood (Foelix 2011, but see exceptions in Kuntner *et al*. 2012). Hence, the exponential increase in their venom yield may have developed to ensure that the spiders reach high levels of venom production after their final moult as subadults.

Despite undergoing a 45-day acclimatization period prior to milking, the mean venom yield from *A. sutherlandi* spiders collected and milked in winter was 62.9% greater than that of spiders collected and milked in autumn (Fig. 2). This finding presents evidence for intraspecific temporal variation in venom yield for a second species of Australian funnel-web spider, in addition to seasonal variation in venom yield previously reported in *A. robustus* (Wiener 1959). The magnitude of temporal increase in *A. sutherlandi* venom yield is also noteworthy; in comparison, the maximum difference between yields of two seasons in *A. robustus* was 45%, between spring and autumn (Wiener 1959). Apart from the Atracinae, temporal effects have also been observed in the venom yield of araneomorphs such as the theridiid *Latrodectus hasseltii*, and ctenids *Phoneutria fera* and *Phoneutria nigriventer* (Bücherl 1953; Wiener 1956; Schenberg & Pereira-Lima 1966). While it is possible

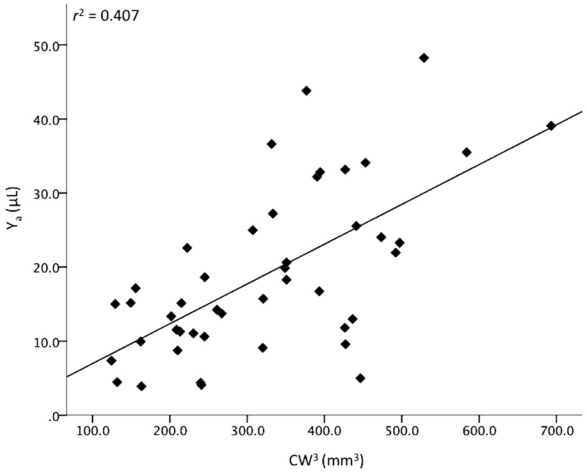

Fig. 1: Relationship between absolute yield (Y_a) and carapace volume (CW^3), which showed the strongest relationship with absolute yield (r^2 = 0.407).

that temporal variation may occur in the venom production of other spiders, considering how infrequently such variation is reported, further investigation is required to substantiate and qualify this phenomenon in nature.

In addition to being scarce, most accounts on temporal variation in spider venom yield are derived from incidental observations made during pharmacological or biochemical studies (e.g. Schenberg & Pereira-Lima 1966). Hence, the evolutionary and ecological underpinnings for such variation remain unknown. While it may be intuitive to attribute the observed variation in *A. sutherlandi* venom yield to plastic responses to seasonality, a common precursor to seasonal polyphenism is the perception of seasonal climatic changes (Azel 2002). However, the acclimatization period (45 days) and controlled microclimatic conditions (temperature, humidity, darkness) used are likely to have precluded the spiders from detecting any climatic shifts following collection from the field. Nevertheless, since the batches were collected in mid-autumn and mid-winter, we are unable to reject the possibility that the spiders had already adjusted their venom production in response to perceived climatic changes before capture, and maintained these adjustments throughout the acclimatization period.

The observed temporal variation in venom yield is not likely to be an artefact of yield differences at the initial time of capture, since a 45-day period is almost certainly sufficient for complete regeneration of venom stores to maximal capacity; Perret (1977) reported that, in other mygalomorphs, regeneration of 50% of original venom supply is achieved within 2–3 days. Furthermore, we observed that, during feeding in the laboratory, like other mygalomorphs, *A. sutherlandi* from both batches primarily immobilized the relatively soft-bodied *T. molitor* larva by physical manipulation (mashing) with their strong chelicerae, as opposed to relying on fang-piercing alone (Wigger, Kuhn-Nentwig & Nentwig 2002). It is thus possible that venom reserves were well conserved during predation over the 45 days; see discussion on the venom optimization hypothesis (Wigger, Kuhn-Nentwig & Nentwig 2002; Morgenstern & King 2013). In addition, even if some venom was utilized for prey submission during the 45-day period, this should not

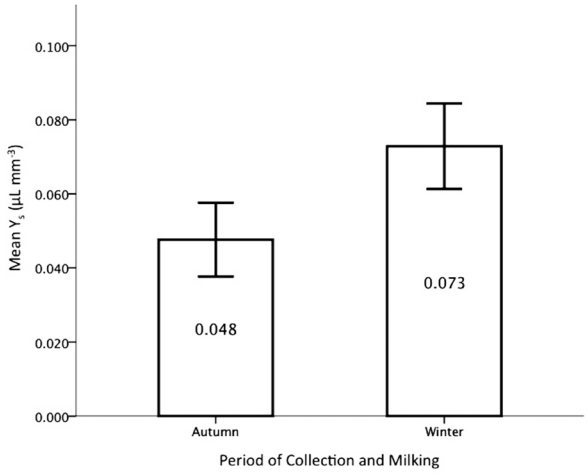

Fig. 2: Temporal variation in venom yields for *A. sutherlandi* collected and milked during autumn and winter. Histogram shows mean values and associated standard errors. Mean size-corrected yield (Y_s) for the winter batch was 62.9% higher than that of the autumn batch.

have affected the observed temporal variation in yield, since the feeding and milking were performed at the same time intervals in both batches.

Perret (1977) also observed the occurrence of moulting in his mygalomorphs to vary with season. Although venom yield of *A. robustus* and *Coremiocnemis tropix* have been shown to decrease prior to moulting (Wiener 1959; Herzig 2010), none of the *A. sutherlandi* used in our study moulted during the 45 day acclimatization period nor in the 30 days following milking (after which the spiders were preserved for size measurement). Furthermore, as adult araneomorphs generally do not moult, seasonal variation in moulting is unlikely to account for temporal variation in venom yield of *Latrodectus* and *Phoneutria* (Bücherl 1953; Wiener 1956; Schenberg & Pereira-Lima 1966).

It is unclear why such a large disparity (62.9%) exists between venom yields of *A. sutherlandi* milked in winter compared to autumn. One explanation may be that the spiders possess mechanisms to increase venom accumulation during winter as a means of capitalizing on their relative inactivity (pers. obs.) in preparation for increased feeding in the coming spring – potentially on prey items that are more difficult to immobilize compared to *T. molitor*. For example, the remains of formicids and coleopterans have been found at *A. sutherlandi* burrow entrances on many occasions (pers. obs.). We propose that future longitudinal comparisons of venom yield and metabolic expenditure as well as prey type and consumption quantity across the seasons would appropriately test this postulation. Indeed, the Atracinae would be ideal study candidates for such pursuit, given the relative ease of venom collection and their extensive (>15 years) lifespans (King, Tedford & Maggio 2002; Morgenstern & King 2013).

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