RESEARCH ARTICLE

Ontogenesis, gender, and molting influence the venom yield in the spider *Coremiocnemis tropix* (Araneae, Theraphosidae)

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ABSTRACT

The demand for spider venom increases along with the growing popularity of venoms-based research. A deeper understanding of factors that influence the venom yield in spiders would therefore be of interest to both commercial venom suppliers and research facilities. The present study addresses the influence of several factors on the venom yield by systematically analyzing the data obtained from 1773 electrical milkings of the Australian theraphosid spider *Coremiocnemis tropix*. Gender and ontogenesis were found to cause a major effect on the venom yield, as adult female *C. tropix* yielded significantly more venom than adult males. During ontogenesis, the venom yield increased with increasing size of the spiders. Furthermore, a significant reduction in the venom yield during the 50-day time interval preceding a molt was found. On the other hand, extended milking intervals (up to 449 days) and different states of nutrition (as an indication of how well the spider was fed) did not significantly affect the venom yield. Overall, the present findings suggest that venom production in spiders is carefully balanced between the demand for venom and the energy costs associated with its production. It can therefore be concluded that, in line with the venom optimization hypothesis, venom is a precious resource for spiders, which have implemented control mechanisms to ensure economical venom production and usage.

KEYWORDS: Spider venom yield, gender, ontogenesis, milking interval, state of nutrition, molt

INTRODUCTION

Spider toxins are becoming increasingly popular for venoms-based drug discovery (Escoubas and King, 2009; Vetter et al, 2010) as evidenced by the exponential increase in spider toxin sequences being deposited in protein and DNA sequence databases (King et al, 2008; Herzig et al, 2010). Spider venoms are extremely complex mixtures (Rash and Hodgson, 2002) and the majority of their effects are caused by short peptide toxins (<10kDa). Some spider venoms might contain several hundred different toxins (Escoubas et al, 2006), implying that the relative amount of each toxin within the venom is fairly low. In terms of toxin discovery, this can be an obstacle for some of the less abundant toxins when it comes to isolating sufficient toxin amounts for sequence determination and toxin characterization. Introduction of new and more sensitive proteomics methods in future will certainly help to improve this problem. Another way to overcome this obstacle is by increasing the venom amounts available for research. This could for example be achieved by providing the optimal conditions for the spiders in order to maximize venom yield.

Despite several thousand papers have been published on spider venoms and toxins, our understanding of the mechanisms involved in venom/toxin production and the factors influencing the venom yield is still rudimentary. By far the most studied factor known to affect venom production in spiders is the gender. As a general rule, female spiders yield more venom than males of the same species (Herzig et al, 2008). The size is also expected to play an important role in the ability of a spider to produce venom, with larger spiders assumed to produce more venom than smaller individuals of the same species. An exponential increase in the venom

yield was previously reported for two araneomorph spider species (Malli et al, 1993; Herzig et al, 2004), but no data have so far been published on the development of venom yield in mygalomorph spiders. The milking interval has also been reported to affect the venom yield in theraphosid spiders, with milking intervals of less than four weeks leading to a reduction in venom yields (Perret, 1977). However, it is unclear whether more extended milking intervals would be beneficial for the venom yield.

An interesting question for anyone trying to optimize venom yield in spiders is how much to feed the spiders. Feeding too often and too much is both time consuming and not economical, whereas feeding not enough might impair venom yield and/or the health of the spider. So far, it has not been examined whether a correlation exists between the amount of food and the resulting venom yield. Another factor that has been almost completely neglected is molting. Molting is a critical and dangerous period for a spider, as it cannot defend itself against predators until the new exoskeleton has sufficiently hardened. Furthermore, the process of forming a new exoskeleton underneath the existing one and the act of molting itself is expected to draw considerable energy resources from the spider. Reducing venom production could therefore be one way of saving energy, which could then be re-directed towards the molting process. However, so far no concrete data have been published on the effect of molting on the venom yield.

In order to study the effect of each of these factors on the venom yield within a single species of spiders, 1773 individually collected electrical milkings of the Australian theraphosid spider *Coremiocnemis tropix* were analyzed. It was anticipated that systematic analysis of these parameters should reveal ways of improving venom yields, which could lead the way to the discovery of new and less abundant spider toxins. In addition, this study might provide insights into the mechanisms that regulate venom production in spiders.

MATERIALS AND METHODS

Spider collection and maintenance
The *C. tropix* specimens used for this study were collected from three different collection sites within 70km from Cairns (Queensland, Australia). Several voucher specimens from each site were determined as *C. tropix* by Robert Raven (Queensland Museum, Brisbane, Australia). All spiders were collected during a single field trip and were then kept at Monash University (Melbourne, Australia) from October 2005 until May 2008. All spiders were then relocated to the University of Queensland (Brisbane, Australia), where they remained for the rest of the study period, up to June 2010. Spiders were always kept in windowless rooms (except for during relocation), and light bulbs or heating cords were used to provide additional heating to simulate the higher temperatures of their natural habitats near Cairns, although the temperature was not monitored.

During the entire study period, all spiders were solely fed on crickets (*Acheta domesticus*), which is the most common food used for theraphosid spiders in captivity. Usually 1-3 crickets were fed to each spider about once every four weeks, but spiders were not fed for at least 1 week prior to

each milking or when they were in the process of molting. In order to prevent dehydration, the spiders were kept on a substrate mixture of potting mix and sand, which was always kept moist. Food debris was removed to avoid fungal infections.

Sex determination and spider size
The sex of each spider included in the present study was determined after reaching the adult stage and then, retrospectively, applied to the venom yield data from previous milkings of juvenile stages of the same individual. Adult males were easily recognized by the presence of palpal bulbs. Females were determined by the presence of a *receptaculum seminis* and they were assumed to be adult after reaching a prosoma length of >10mm, which was the size of the smallest females that have been observed with egg-sacs. In contrast to araneomorph spiders, adult mygalomorph females continue to molt several times during their adulthood, which means that their size can still increase after reaching adulthood. Consequently, the size of adult female mygalomorph spiders can vary considerably.

One of the aims of the present study was to compare the venom yield across different size classes. Therefore, a measurement for the size of the spiders was required. An obvious measurement would be the body size, *i.e.*, the length of the prosoma plus the length of the opisthosoma. One major drawback of using the body size is that the length of the opisthosoma can vary considerably in the same spider between consecutive molts, depending on how well the spider is fed. Another possible measurement for the spider size would be the legspan, but most spiders (including *C. tropix*) exhibit sexual dimorphism, with males having a larger legspan than females of equal body size (Herzig and Hodgson, 2009). Therefore, the length of the prosoma, which remains constant between molts, was used in the present study to define different size classes of spiders.

Venom collection
Venom was collected by using electrical stimulation ('milking') according to a recently described method (Herzig and Hodgson, 2009). All milkings were carried out between October 1, 2005 (*i.e.*, about 3 weeks after the collection of the spiders from their natural habitats) and June 10, 2010. In total, 1773 milkings (1127 female, 646 male) were carried out using 130 individual *C. tropix* (71 female, 59 male). All venoms were freeze-dried after the collection and only the dried venom mass was used for statistical analysis. Unless otherwise stated, all venom yields refer to the freeze-dried venom mass in milligram.

Data analysis
The statistics function in SigmaPlot 11.0 (Systat Software Inc.) was used for all statistical tests. Due to the fact that the venom yield data were not normally distributed, only non-parametric statistics were used.

RESULTS

Spider and venom data
Preliminary analysis (data not presented) indicated that there was no significant difference in the venom yield between the three collection sites. Hence, the data from all collection

sites were pooled for the subsequent data analysis. The largest specimen of *C. tropix* used in this study had a prosoma length of 17mm and the maximum venom yield observed for a single milking was 27.07mg (fresh, liquid) or 5.46mg (freeze-dried), respectively. Most of the spiders were milked repeatedly and the maximum number of subsequent milkings per individual was 36 (for two individuals). In addition, there were 38 spiders that survived 20 or more subsequent milkings. From a total number of 1773 milkings analyzed for the present study, an average of 79% yielded venom.

Ontogenesis and intersexual variations
In order to study this parameter, 1121 milkings from 71 female and 646 milkings from 59 male *C. tropix* across several size ranges (*i.e.*, from juvenile to adult) were analyzed.

The present results (Figure 1) indicate a significant effect of ontogenesis on venom yield in both female and male *C. tropix* (P <0.001 each, according to a Kruskal-Wallis ANOVA on ranks). Independently of the spider's gender, the venom yield increased with increasing prosoma size, while the percentage of spiders that did not yield any venom decreased. In addition, a comparison of the venom yield between males and females using Mann-Whitney rank sum tests showed no significant differences for the prosoma size-classes 4-6mm (*i.e.*, 4.0-5.9mm prosoma length) and size-class 6-8mm (P = 0.897 and 0.738, respectively), but significant differences for the larger size classes, *i.e.*, 8-10mm (P = 0.006), 10-12mm (P <0.001) and 12-14mm (P = 0.046).

Milking interval
For data analysis, all milkings carried out at a wide range of time intervals 18 to 449 days were arranged in 50-day intervals. Based on the observation that both size and gender can significantly influence the venom yield, only data from the size class with the largest number of milkings from each gender were used. Hence, 322 milkings from 58 adult females (size class 12-14mm) and 142 milkings from 38 adult males (size class 10-12mm) were analyzed.

For both female (P = 0.075) and male (P = 0.765), a Kruskal-Wallis ANOVA on ranks did not reveal any significant effect of the milking intervals on the venom yield (Figure 2). In addition, there was no clear effect of different milking intervals on the percentage of spiders that did not yield venom.
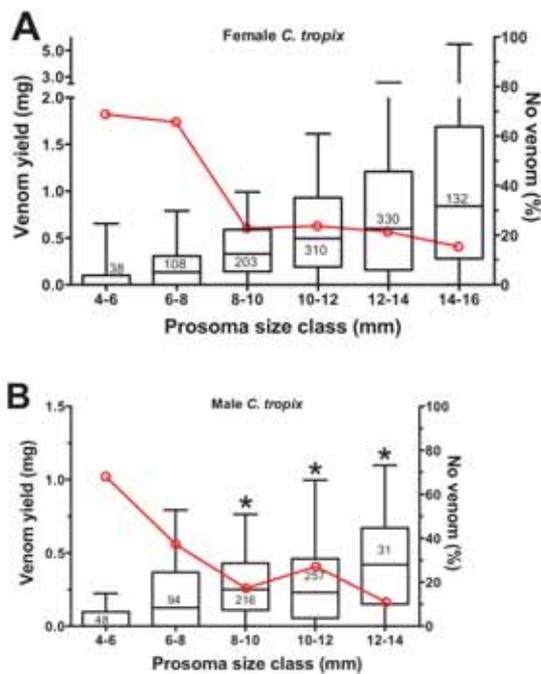

Figure 1. Ontogenetic and intersexual effects. The venom yield in female (A) and male (B) *C. tropix* increases during ontogenesis. In addition, male *C. tropix* of size-classes 8-10, 12-14 and 14-16mm yielded significantly less venom than equal-sized females (*P <0.05). The spiders have been categorized into different size classes according to their prosoma length (*i.e.*, '4-6' = 4.0 to 5.9mm; '6-8' = 6.0 to 7.9mm, *etc*). The box-and-whisker plots indicate the median venom yield in mg of freeze-dried venom (left Y-axis) by the line within the box, the 25th and the 75th percentile (*i.e.*, the bottom and top lines of the box) as well as the 5th and 95th percentile by the whiskers. The number of milkings analyzed for each size class is shown. The red line indicates the percentage of milkings that did not yield any venom (right y-axis).
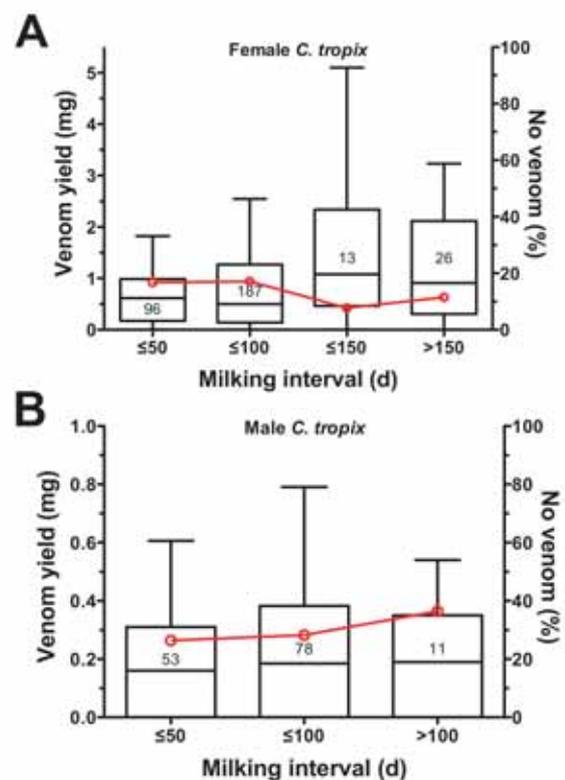
Figure 2. Extended milking intervals. Extended milking intervals did not significantly affect the venom yield. The milkings have been categorized into different 50-day milking intervals and the respective data for the freeze-dried venom yield (left y-axis) is presented in a box-and-whisker plot. Only data from adult female (12-14mm prosoma length) and from adult male (10-12mm prosoma length) was used. The number of milkings analyzed for each milking interval is shown. The red line indicates the percentage of milkings that did not yield any venom (right y-axis).

State of nutrition

For the present study, the 'state of nutrition' is defined as the long-term nutritional status of a spider ranging from starving to extremely well-nourished. While the state of nutrition is expected to be affected by the consumed amount of food and its nutrient composition, recording the amount of food (*i.e.*, number and weight of crickets) for each spider was not a viable option due to the large number of spiders and the long duration of the present study. Another way to determine the state of nutrition was therefore established. Depending on how well each spider was fed the opisthosoma can increase or decrease in size between molts, while the size of the prosoma remains constant. Although the opisthosoma will increase in each dimension when the spider feeds, most of the increase will be seen in the length, as the opisthosoma in *C. tropix* is rather long and narrow than spherical. Therefore, the ratio of opisthosoma length/prosoma length (henceforth called the o/p ratio) should provide an indication of a spider's state of nutrition. However, one might argue that a decrease in the length of the opisthosoma does not necessarily indicate a poor state of nutrition, as it can also be caused by old age or disease (*e.g.*, parasites, fungal infections, *etc*). Data from all spiders that showed signs of impaired health and/or unusual behavior (*e.g.*, as a result of disease or old age) were therefore excluded from the study. Furthermore, it has to be kept in mind that body proportions (prosoma vs opisthosoma lengths) may not be constant during a spider's growth, which would make it difficult to compare the o/p ratios of different-sized spiders. A preliminary analysis of the o/p ratios across all size classes was therefore carried out and it was found that the average o/p ratio decreased with increasing spider size ($P < 0.001$ for both sexes, data not shown), which supports an allometric rather than a proportional growth in *C. tropix*. In order to minimize the effects of the allometric growth, the influence of the state of nutrition on the venom yield was studied in adult spiders of only one size class. To allow for a maximum number of data, the size class 12-14mm prosoma length was chosen for female *C. tropix* (containing 330 milkings of 58 spiders), while the data of size class 10-12mm prosoma length (containing 146 milkings of 39 spiders) was used for males.

A Kruskal-Wallis ANOVA on ranks did not show any significant effect of the state of nutrition on the venom yield in female ($P = 0.815$) and male ($P = 0.225$) *C. tropix* (Figure 3). Independent of the sex of the spiders, an increase of the numbers of spiders that did not yield venom was observed towards very high and low o/p ratios.

Molting

To study possible effects of molting on venom yield the following parameters were analyzed: (i) the effect on venom yield of the time from last molt based on 309 milkings from 58 adult females (12-14mm prosoma length) and 146 milkings from 39 adult males (10-12mm prosoma length); (ii) the effect on venom yield of the time to next molt based on 226 milkings from 38 adult females (12-14mm prosoma length).

A Kruskal-Wallis ANOVA on ranks did not reveal any significant effect of the time from last molt on the venom yield in female *C. tropix* ($P = 0.373$), while a trend of reduced venom yield with increasing time from their last molt was found for males ($P = 0.066$) (Figure 4). A similar analysis for the time to the next molt in females indicated a significant effect on venom yield ($P = 0.026$). Post-hoc comparison using the data from over 300 days before the next molt interval as reference (as this is assumed to be the least influenced by any molting related effects) showed that the venom yield in the last 50 days prior to a molt was significantly reduced ($P < 0.05$). In addition, a maximum in the percentage of milkings that did not yield venom was observed during this interval.

DISCUSSION

Spider and venom collection

A possible concern relating to the venom yield is that maintenance of the spiders could have resulted in the loss of venom. All spider maintenance was therefore carried out with minimal disturbance to the spider to ensure that no venom was released for defensive purposes. Australian funnel web spiders of the genera *Atrax* and *Hadronyche* are well known to respond to disturbance by rearing of the front two pairs of legs and presenting the spread fangs with a droplet of venom on each fang (Wiener, 1957). Rearing of the legs and the presentation of the spread fangs was also observed in some *C. tropix* after aggravation, but this behavior never
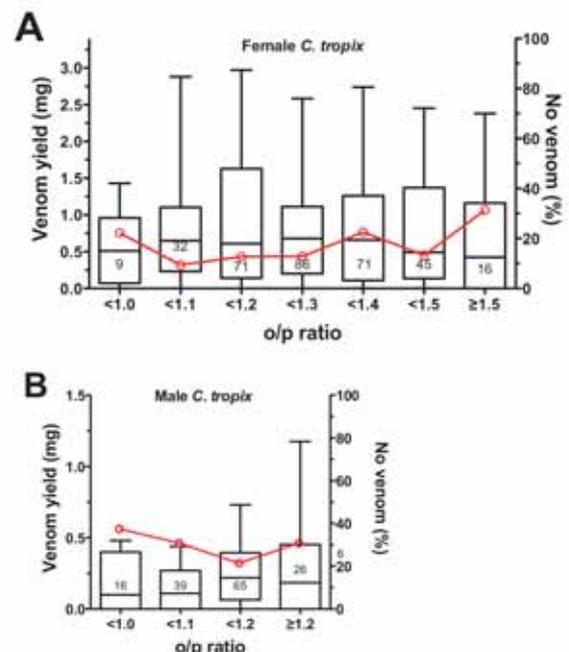

Figure 3. State of nutrition. The state of nutrition (as an indication of how well each spider was fed) had no significant effect on the venom yield (left y-axis) in female (A) and male (B) *C. tropix*. The o/p ratio is calculated by dividing the length of the opisthosoma by the length of the prosoma. The milking data have been categorized in different o/p ratio classes (<1 = 0.90 to 0.99; <1.1 = 1.00 to 1.09; *etc*). Only data from adult female (12-14mm prosoma length) and from adult male (10-12mm prosoma length) was used for analysis and the data are presented as a box-and-whisker plot. The number of milkings analyzed per o/p ratio class is shown. The red line indicates the percentage of milkings that did not yield any venom (right y-axis).
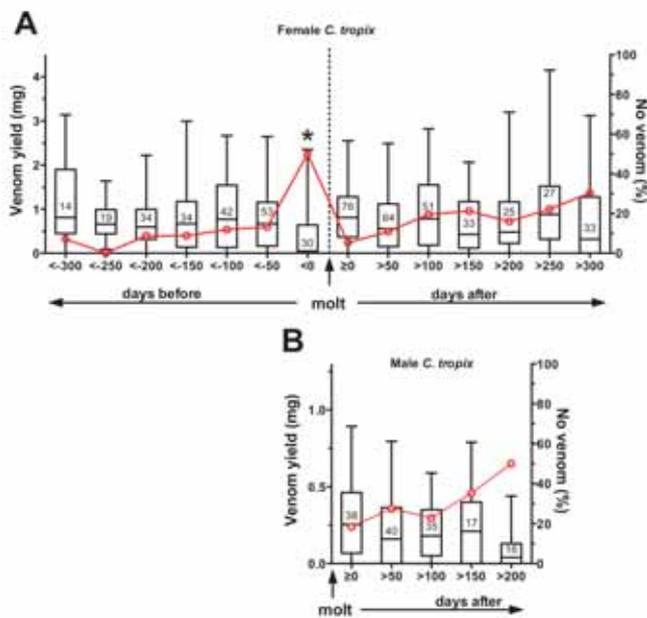
Figure 4. Effects of molting. A significant reduction in the venom yield in female *C. tropix* (A) was found in the time interval immediately preceding the molt (*P <0.05), while no significant effect was observed in the time after the molt in both females and males (B). The venom yield data (left Y-axis) has been categorized according to different intervals to the next molt or from the last molt (<0 = -1 to -50 days; <-50 = -51 to -100 days; ≥0 = 0 to 50 days; >50 = 51 to 100 days; *etc*) and presented as a box-and-whisker plot. Only data from adult female (12-14mm prosoma length) and from adult male (10-12mm prosoma length) was used. The number of milkings analyzed per time interval is shown. The red line indicates the percentage of milkings that did not yield any venom (right Y-axis), which peaked at the time interval immediately preceding the molt.

included the voluntary release of any venom. It is therefore unlikely that spider maintenance had any negative impact on venom yield.

The fact that the subset of data used for the present study was much larger than the one used in our previous study (Herzig and Hodgson, 2009) might explain why the maximum prosoma length and venom yields observed were higher in the present study. Furthermore, two spiders survived 36 milkings and several others survived more than 20 milkings (and are alive to date) and their venom yield did not decrease during repetitive milkings (data not shown), which underlines that the applied electrical stimulation method was well tolerated by the spiders. In addition, due to the electrical stimulation, the spider looses voluntary control over the venom secretion, suggesting that all available venom will actually be secreted. In turn, if no venom is collected after electrical stimulation, it must be concluded that the spider did not have any venom at the time of milking. The fact that 21% of all spiders did not yield any venom implies that one or several factors must exist that can completely abolish venom production. However, at this point, the nature of those factors can only be speculated. Nevertheless, this lack of venom in about one fifth of all milkings might explain the occurrence of part of the so-called 'dry' bites reported

from spiders (Isbister, 2004). Another part of the dry bites might be explained by the voluntary decision of the spider not to secrete venom during a bite, as a 'venom-less' bite might also be quite efficient for defensive purposes and it would save the spider the energy required to re-synthesize the venom.

Ontogenesis and intersexual variations

Based on our previous findings, it was anticipated that female *C. tropix* yield significantly more venom than males and that larger spiders yield more venom than smaller specimen (Herzig and Hodgson, 2009). The present results confirmed our previous findings, although there were some surprises. For example, there was no significant intersexual difference in the venom yield of the smaller spiders with a prosoma length of less than 8mm. Interestingly, the size class 8-10mm in which intersexual differences in the venom yield were first observed nicely correlates with the appearance of adult males. The smallest adult male recorded in the present study had a prosoma length of 8.1mm. Hence, it might be concluded that juvenile *C. tropix* increase their venom yield independently of the gender, whereas the increase in venom production is reduced in adult males as compared to adult females. One reason might be that in order to reach adulthood, males have to spent more energy for the changes that occur during their final molt, such as the development of palpal bulbs, and one way of saving energy is by reducing venom production. After reaching adulthood, male spiders then require lower venom quantities than females, as their food intake is considerably lower, so they maintain their venom production at a lower level compared to females. In contrast, female mygalomorph spiders require more food as they are larger in body size (in most species), live considerably longer and they have to produce eggs, construct the egg-sac, molt, and then continue the reproductive cycle with other males in subsequent years. The observation that female spiders yield more venom than their male counterparts was already shown in several species by many different groups, *e.g.*, in *Atrax robustus* (Wiener, 1959), *Cupiennius salei* (Kuhn-Nentwig et al, 2004), *Missulena bradleyi* and *M. pruinosa* (Herzig et al, 2008), *Loxosceles reclusa* (Morgan, 1969; Morris and Russell, 1975), *L. intermedia* (De Oliveira et al, 1999), *Phoneutria nigriventer* (Herzig et al, 2002), *Stromatopelma calceatum griseipes* (Celerier et al, 1993), *Tegenaria agrestis* and *T. duellica* (Binford, 2001); all species names according to Platnick (2010).

It has recently been shown that intra-egg-sac stages of *P. nigriventer* already have a completely formed venom apparatus and transcripts of a vertebrate-active neurotoxin were also present (Silva et al, 2010). What still needs to be addressed is how the venom yield and composition develops during the ontogenesis of spiders. While the venom composition is out of the scope of the present study, the venom yield in female *C. tropix* increased linearly during the spider's growth. This is in contrast to the exponential growth previously reported for the araneomorph spiders *Cupiennius salei* (Malli et al, 1993) and *Phoneutria nigriventer* (Herzig et al, 2004). One reason could be that araneomorph and mygalomorph spiders differ in the actual position/size of the venom gland. In mygalomorph spiders, the venom gland is located in the basal part of the chelicerae, whereas it extends into the prosoma in araneomorph spiders. Hence, a

similar-sized araneomorph spider would be expected to have a larger venom gland than a mygalomorph spider. Another factor that could play a role is that araneomorph spiders do not molt after reaching adulthood, whereas mygalomorph spiders continue molting during adulthood. Hence, it is possible that araneomorph spiders have developed an exponential increase of their venom yield to ensure that they reach a high level of venom production at adulthood (*i.e.*, after their final molt), whereas mygalomorph spiders continuously increase their venom yield while they grow (even during adulthood). However, based on the rather limited data available on this topic, it is impossible to conclude at the present stage whether the observed differences are due to general differences between araneomorph and mygalomorph spiders or rather caused by family-dependent mechanisms, as *C. tropix* belongs to the family Theraphosidae, whereas both *C. salei* and *P. nigriventer* belong to the family Ctenidae. The venom yield in male *C. tropix* also increased during growth, but the increase was not as steep an in females. Overall, it can therefore be concluded that the venom yield in *C. tropix* increases with increasing size of the spider, which might be explained by the increase in venom gland size and capacity. This makes sense in terms of ecology, since larger spiders tend to overcome larger prey, which in turn requires larger amounts of venom. The spiders in the present study were usually fed with crickets of the appropriate size (*i.e.*, the maximum body length of the cricket did not exceed the spider's body length). When feeding larger crickets to juvenile *C. tropix*, it was observed that the spiders tend to avoid or even escape the cricket. This could imply that spiders can somehow 'judge' the maximum prey size they can overwhelm by using their physical strength, their venom, or a combination of both. Wigger et al (2002) already demonstrated that spiders inject more venom into prey that is more difficult to overwhelm, suggesting that the spiders are able to make some kind of judgment about their prey.

Another unprecedented observation was that the percentage of spiders that did not yield any venom increased in smaller specimen of *C. tropix*. This might be partially explained by limitations of the applied venom extraction method that requires the spiders to be of a certain size that allows handling and applying the electric shock with the forceps. In addition, one could argue that smaller spiders received a comparably larger electrical stimulation, which might have resulted in a decreased venom yield. However, this hypothesis is unlikely as the voltage used for the electrical stimulation was modified between 9 and 12 volts based on the size of the spider (Herzig and Hodgson, 2009), with smaller spiders receiving a weaker electric shock. Another limitation could be the accuracy of 0.01mg and 0.1mg of the different scales used to determine the venom yields, which would have a larger impact on small venom amounts yielded by smaller spiders. However, the fact that some smaller-sized spiders yielded considerable venom amounts (up to 0.7mg in the 4-6mm size class) argues against general methodological reasons for the observed difference. Another explanation could be by assuming that the venom yield in the time interval before a molt is reduced (see separate discussion on 'molting' below). As the frequency of molting is higher in smaller spiders, the average number of spiders being in a pre-molting state would be higher, which in turn could explain the larger percentage of smaller spiders that did not

any yield venom. Overall, it is concluded that (independent of the gender of a spider) the proportion of spiders that yield venom increases during growth.

Milking interval

While Perret (1977) already demonstrated that reducing the milking intervals decreased the venom yields in theraphosid spiders, the present study focused on the effect of extended milking intervals. In the present study, milking intervals failed to show a significant effect on the venom yields, although a trend towards higher venom yields with longer milking intervals was observed in females. Despite this trend, the present results suggest that in order to maximize the venom yield, more frequent milkings at intervals <50 days would be more efficient than using less frequent milkings at more extended time intervals. However, the shortest milking interval used in the present study was 18 days and all milkings were grouped in 50-day intervals. Thus, it cannot be excluded that reducing the milking intervals below 50 days will affect the venom yield. According to the results from Perret (1977) it would even be expected that shorter milking intervals decrease the venom yield. Based on the data available for theraphosid spiders, monthly milkings would appear to be a good choice if one mainly aims at maximizing the overall venom yield. However, the fact that longer milking intervals had no significant effect on venom *quantity* does not necessarily imply that the venom *composition* remained unchanged. In fact, it has been reported that newly regenerated venom has a lower protein concentration and exhibits lower toxicity (Boeve et al, 1995). The same authors also reported that emptied glands regenerate rapidly an important part of their venom quantity, whereas venom toxicity only increased slowly. Furthermore, some components of theraphosid venoms such as hyaluronidase regenerate at a slower rate than others (*e.g.*, proteins, free amino acids) and inter-specific variations in the speed of venom regeneration were also reported (Perret, 1977). Due to the variations in the regeneration time for different venom components, the possibility exists that monthly milking intervals might be too short to regenerate all venom components (despite the fact that most of the venom quantity will likely have been regenerated during this period).

State of nutrition

The present results demonstrate that a change in the long-term state of nutrition (as expressed in the o/p ratio) does not significantly affect the venom yield. However, towards very high and low o/p ratios, there seems to be an increase of spiders that do not yield any venom. This might be explained in two ways; badly-nourished spiders (with a very low o/p ratio) do not have sufficient energy resources for venom synthesis, whereas extremely well-nourished spiders (with a very high o/p ratio) have a decreased demand for venom, as they do not need to capture prey for some time. In conclusion, excessive feeding is not beneficial for increasing the venom yield. In terms of optimizing the venom production, some maintenance time and feeding costs might be saved by providing only the minimum amount of food that is required to maintain a good health of the spiders. Based on present data, an o/p range 1.0-1.2 would be recommended for *C. tropix*, as this also minimizes the numbers of spiders that did not yield any venom. Another interesting question is whether the type of food might influence the venom yield.

However, as all *C. tropix* used in the present study were solely fed on crickets (*Acheta domesticus*), no conclusion about the influence of the food type can be drawn. Additional experiments are therefore required to determine any influence of the type of diet or nutrient composition on the venom yield in spiders.

Molting

The present study clearly shows a significant decrease in the venom yield during the 50-day interval before a molt, whereas any of the other intervals (either before or after a molt) had no significant effect on the venom yield. The main reason for the venom yield being reduced prior to molt is the fact that half of the spiders did not yield any venom. Overall, these data are partially in line with the observation made by Wiener (1959) that no venom was obtainable from *A. robustus* a few days prior to molting, implying that this phenomenon is not restricted to *C. tropix* but more likely a general phenomenon in (at least mygalomorph) spiders. It might further explain part of the 21 percent of milkings that did not yield any venom and part of the 'dry-bites' reported from spiders (Isbister, 2004). It would be interesting to see at which time in the 50-day interval preceding the molt the reduction in the venom yield starts to occur. The present data suggests that 30-days prior to the molt are critical, however, the number of milkings is too low to allow for a meaningful statistical comparison of shorter time intervals. More detailed experiments focusing only on this aspect would therefore be required. It would be expected that spiders minimize the time interval with reduced venom stocks, as even under conditions of reduced food intake some venom might still be required for defensive purposes against intra-specific aggression and/or predators.

In venoms of both sexes, the percentage of spiders that did not yield venom increased with increasing time from a molt. However, a gender-dependent explanation is suggested. With increasing time from a molt, the time to the next molt decreases, which in female *C. tropix* results in an increase in the percentage of spiders that are in the 50-day interval before a molt (i.e. the subsequent molt). In adult males (which do not molt anymore) the increase in the percentage of spiders that did not yield venom might be attributed to ageing of the males, as they only live for a few months after their final molt. Another interesting observation was made on five female *C. tropix* milked within the first day after a molt (data not shown), with two of them yielding a decent amount of dried venom (1.6mg each). This indicates that even shortly after molting, reasonable amounts of venom can be obtained. This further supports the findings of Wiener (1959) reporting that after aggravation *A. robustus* showed drops of venom on the fang-tips only 3hr after a molt. The same spider was able to bite a mouse one day after a molt, which died 20min later, implying that the venom contained the active component and that it has been administered to the mouse. In conclusion, the venom amount is decreased prior to a molt but readily available shortly after a molt. It therefore seems to be uneconomical to carry out venom extractions on spiders that are close to molting. A good indication of an upcoming molt can be the time that has passed since the last molt (usually, adult female theraphosids molt about once every year or once in two years in very old specimen) and if the spider stops feeding.

CONCLUSIONS

Of all the factors that were examined in this study, gender and ontogenesis have the greatest impact on venom yield. In addition, the venom yield was reduced prior to a molt. On the other hand, factors such as extended milking intervals and the state of nutrition had no significant effect on venom yield. Overall, these findings support the conclusion that venom production in *C. tropix* is subject to demand. Larger spiders that require more food have an increased venom yield, whereas when the demand is low, such as prior to a molt or in adult males, the venom production is reduced. The obvious purpose of these regulatory processes is to save energy. Although the energy costs associated with venom production have not been studied in spiders, they are assumed to be considerable as indicated by a recent study on scorpions which showed that the metabolic rate during the first 72hr after milking is 39% higher in milked than in unmilked scorpions (Nisani et al, 2007). Regulatory processes that control venom production in spiders would complement the venom optimization theory on the economical usage of venom in spiders as proposed by Nentwig and colleagues. They concluded that spiders 'know' how much venom is in their venom glands and that they choose their prey items accordingly (Wullschleger and Nentwig, 2002). In addition, they demonstrated that more venom is used for prey species that are more difficult to overwhelm or that could even endanger the spider (Wigger et al, 2002). Overall, venom seems to be a precious resource for spiders. Therefore, control mechanisms are implemented that ensure its most economical production and usage.

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STATEMENT OF COMPETING INTERESTS

None declared.

REFERENCES

Binford GJ. 2001. An analysis of geographic and intersexual chemical variation in venoms of the spider *Tegenaria agrestis* (Agelenidae). Toxicon, 39, 955-968.

Boeve J-L, Kuhn-Nentwig L, Keller S and Nentwig W. 1995. Quantity and quality of venom released by a spider (*Cupiennius salei*, Ctenidae). Toxicon, 10, 1347-1357.

Celerier ML, Paris C and Lange C. 1993. Venom of an aggressive Theraphosidae (*Scodra griseipes*): milking the venom, a study of its toxicity and its characterization. Toxicon, 31, 577-590.

De Oliveira KC, De Andrade RMG, Giusti AL, Da Silva WD and Tambourgi DV. 1999. Sex-linked variation of *Loxosceles intermedia* spider venoms. Toxicon, 37, 217-221.

Escoubas P, Sollod B and King GF. 2006. Venom landscapes: Mining the complexity of spider venoms via a combined cDNA and mass spectrometric approach. Toxicon, 47, 650-663.

Escoubas P and King GF. 2009. Venomics as a drug discovery platform. Expert Rev Proteomics, 6, 221-224.

Herzig V, Ward RJ and dos Santos WF. 2002. Intersexual variations in the venom of the Brazilian 'armed' spider *Phoneutria nigriventer* (Keyserling, 1891). Toxicon, 40, 1399-1406.

Herzig V, Ward RJ and dos Santos WF. 2004. Ontogenetic changes in *Phoneutria nigriventer* (Araneae, Ctenidae) spider venom. Toxicon, 44, 635-640.

Herzig V, Khalife AA, Chong Y et al. 2008. Intersexual variations in Northern (*Missulena pruinosa*) and Eastern (*M. bradleyi*) mouse spider venom. Toxicon, 51, 1167-1177.

Herzig V and Hodgson WC. 2009. Intersexual variations in the pharmacological properties of *Coremiocnemis tropix* (Araneae, Theraphosidae) spider venom. Toxicon, 53, 196-205.

Herzig V, Wood DLA, Newell F et al. 2010. ArachnoServer 2.0, an updated online resource for spider toxin sequences and structures. Nucleic Acids Res, doi 10.1093/nar/gkq1058.

Isbister GK. 2004. Mouse spider bites (*Missulena spp.*) and their medical importance. A systematic review. Med J Aust, 180, 225-227.

King GF, Gentz MC, Escoubas P and Nicholson GM. 2008. A rational nomenclature for naming peptide toxins from spiders and other venomous animals. Toxicon, 52, 264-276.

Kuhn-Nentwig L, Schaller J and Nentwig W. 2004. Review: Biochemistry, toxicology and ecology of the venom of the spider *Cupiennius salei* (Ctenidae). Toxicon, 43, 543-553.

Malli H, Vapenik Z and Nentwig W. 1993. Ontogenetic changes in the toxicity of the spider *Cupiennius salei* (Araneae, Ctenidae). Zool Jb Physiol, 97, 113-122.

Morgan PN. 1969. Preliminary studies on venom from the brown recluse spider *Loxosceles reclusa*. Toxicon, 6, 161-165.

Morris JJ and Russell RL. 1975. The venom of the brown recluse spider *Loxosceles reclusa*: composition, properties and improved method of procurement. Fed Proc, 34, 225.

Nisani Z, Dunbar SG, and Hayes WK. 2007. Cost of venom regeneration in *Parabuthus transvaalicus* (Arachnida: Buthidae). Comp Biochem Physiol A, 147, 509-513.

Perret BA. 1977. Venom regeneration in tarantula spiders-I. Analysis of venom produced at different time intervals. Comp Biochem Physiol, 56A, 607-613.

Platnick NI. 2010. The world spider catalog, version 10.5. American Museum of Natural History, online at http://research.amnh.org/entomology/spiders/catalog/index.html.

Rash LD and Hodgson WC. 2002. Pharmacology and biochemistry of spider venoms. Toxicon, 40, 225-254.

Silva LM, Fortes-Dias CL, Schaffert PP et al. 2010. Developmental biology of the Brazilian 'Armed' spider *Phoneutria nigriventer* (Keyserling, 1891): Microanatomical and molecular analysis of the embryonic stages. Toxicon, in press.

Vetter I, Davis JL, Rash LD et al. 2010. Venomics: a new paradigm for natural-products-based drug discovery. Amino Acids, doi 10.1007/s00726-010-0516-4.

Wiener S. 1957. The Sydney funnel-web spider (*Atrax robustus*): I Collection of venom and its toxicity in animals. Med J Aust, 44, 377-383.

Wiener S. 1959. The Sydney funnel-web spider (*Atrax robustus*): II Venom yield and other characteristics of spider in captivity. Med J Aust, 46, 678-682.

Wigger E, Kuhn-Nentwig L and Nentwig W. 2002. The venom optimisation hypothesis: a spider injects large venom quantities only into difficult prey items. Toxicon, 40, 749-752.

Wullschleger B and Nentwig W. 2002. Influence of venom availability on a spider's prey-choice behaviour. Functional Ecology, 16, 802-807.