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Influences on venom yield in Australian tigersnakes (*Notechis scutatus*) and brownsnakes (*Pseudonaja textilis*: Elapidae, Serpentes)

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Abstract

The rates at which venomous animals produce venoms are of obvious biological and medical importance, but factors influencing those rates remain poorly understood. We gathered data on venom yield (wet mass of venom) and percentage solids (dry mass of the venom divided by wet mass) for 53 eastern brownsnakes (*Pseudonaja textilis*) and 36 mainland tigersnakes (*Notechis scutatus*) over a 4-year period at Venom Supplies Pty. Ltd, a commercial venom production facility in South Australia. Tigersnakes yielded about threefold more venom (by wet mass) than brownsnakes, but with slightly lower percentage solids. Both species showed significant geographic variation in percentage solids. Venom yields varied as a function of the snake's sex and geographic origin, but these effects were secondary consequences of geographic and sex-based differences in body size. Relative head size affected venom yield in brownsnakes but not tigersnakes. Overall, the amount of venom that a snake produced during milking was affected by its species, its geographic origin, its body size and relative head size, and by the time of year that it was milked, as well as by interactions among these factors. Body size was the most important effect on venom yield, with yields increasing more rapidly with size in brownsnakes than in tigersnakes. Research at the intersection of snake ecology and venom characteristics has great potential, but will require a genuinely interdisciplinary approach.

Keywords: Venom; Snake size; Head size; Percentage solids; *Notechis scutatus*; *Pseudonaja textilis*

1. Introduction

Uniquely among the continents of the world, the majority of snake species found within Australia are venomous. Front-fanged (proteroglyphous) snakes of the family Elapidae have undergone a major adaptive radiation within Australia, and are abundant over most of the continent (Shine, 1991a; Keogh et al., 1998, 2000). Consequently, these animals have attracted considerable research interest. The scientific literature on Australian elapids (like that on venomous snakes worldwide) is however, divided into two virtually separate parts, with little contact between them. Some researchers have focused on the ecology and evolution of Australian proteroglyphs, others on the pharmacological and medical aspects of venoms. Despite some worthy attempts to integrate the two approaches (e.g. Cogger, 1971; Sutherland, 1983; Sutherland and Tibballs, 2001), most scientists have chosen to work on either the snakes or their venoms, but not both.

Although it has not been a popular topic of research, the intersection between the ecology of snakes and the production of venom can provide many insights (Daltry et al., 1997). For example, variation in venom composition related to a snake's size, sex or geographic location may reflect the ways that dietary habits shift with these factors (e.g. Daltry et al., 1997; Greer, 1997); and geographic variation in venom components may elucidate phylogenetic relationships or taxonomy (Okuda et al., 2001). Studies on

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the relative toxicity of venoms to natural prey species as well as to laboratory rodents (Broad et al., 1979; Minton and Minton, 1981; da Silva and Aird, 2001) have great potential to elucidate reasons for interspecific variation in venom characteristics. In the present paper, we focus on factors that influence the amount of venom that a snake yields when milked: a topic that, although undoubtedly relevant to both the biology of snakes and the consequences of snakebite, has attracted surprisingly little rigorous study.

The amount of venom that a snake produces can clearly influence the severity of envenomation following a bite, although in many cases snakes will allocate only a fraction of their existing venom reserves to any single strike (Fairly and Splatt, 1929; Morrison et al., 1982, 1983; Young and Zahn, 2001). Venom production has strong ecological implications also; because it is a complex mixture of compounds and presumably metabolically expensive to produce, we would expect natural selection to fine-tune that rate of production (or perhaps, the amount of venom carried within the venom gland). Whilst there is disagreement on whether geographical venom component variation within species is due to prey variation or time of isolation (Williams et al., 1988; Daltry et al., 1996; Mebs, 1999), venom yield might also be expected to shift with such factors in the snake's biology, to enable them to produce enough venom to subdue the numbers, kinds and sizes of prey that they are likely to encounter. Because such rates of encounter with different types of prey may vary considerably among snakes from different species, different habitats, different sexes and different body sizes (Daltry et al., 1997; da Silva and Aird, 2001), we might expect that such factors will also influence the rate at which venom is produced. This prediction remains largely untested. Although species-level mean values and ranges for venom yield per bite are frequently reported, the factors that influence a snake's venom yield appear to have attracted surprisingly little quantitative analysis.

What factors might we expect to influence venom yield? Obvious possibilities include: (1) species; (2) geographic location within a single species; (3) body size; (4) relative head (and thus, venom gland) size; (5) sex; and (6) season. In the present study, we use an extensive data set on venom yield by two species of Australian elapid snakes, based on long-term captives, to clarify the relative importance of these factors as influences on venom yields during 'milking'.

2. Materials and methods

2.1. Study species

Tigersnakes (*Notechis scutatus*) and eastern brownsnakes (*Pseudonaja textilis*) have been responsible for a high proportion of all human and domestic animal snakebites and fatalities in Australia (Mirtschin and Davis, 1992; Mirtschin et al., 1998; Cogger, 2000; Sutherland and Tibballs, 2001). Both are large (to >2 m) terrestrial elapid snakes. Tigersnakes are heavy-bodied, and generally found near wet areas or watercourses; this species feeds primarily on anurans in mainland areas, but island populations have more diverse diets that often include seabirds (Shine, 1977, 1987; Schwaner and Sarre, 1990). Eastern brownsnakes are slender-bodied diurnally-active foragers often abundant in disturbed habitats and agricultural land. Adult brownsnakes feed primarily on introduced house-mice (*Mus domesticus*), but juveniles (and adults in less disturbed habitats) frequently take a broader range of reptile and amphibian prey (Shine, 1977, 1989).

2.2. Methods

Brownsnakes were collected from the wild by hand over the period 1990–2001 from the Barossa Valley and Adelaide regions (SA) and near the Gold Coast in Queensland (QLD). Tigersnakes were collected from three areas: (1) Lake Alexandrina at the mouth of the river Murray in South Australia; (2) Melbourne and environs in Victoria; and (3) the south-eastern part of South Australia (near Penola and Mt Gambier). Nineteen percent of the tigersnakes used were captive bred from parents from the above regions.

The Barossa Valley and Adelaide region has open plains, watercourse habitat and low eucalypt woodlands. The Gold Coast region comprises coastal woodland and forest. The Lake Alexandrina area comprises flood-lands with low shrubs and lignum bushes. The south-east of South Australia consists of woodlands and coastal heath. Melbourne and environs has woodlands and watercourse habitats. Most of these habitats have been substantially modified for agricultural and urban use.

All snakes were maintained at Venom Supplies Pty. Ltd near Tanunda, South Australia (34°36′E and 138°57′S), in individual laboratory cages ranging from $300 \times 450 \times 250$ to $880 \times 600 \times 580$ mm^3 or in open outside 5 m square pits. The cages were placed on racks within a single room (15.0×7.2 m^2), exposed to natural daylight and with temperatures averaging 19 to 36 °C in summer, and 16–28 °C in winter. In the pits, summer temperatures ranged from 9 to 42 °C and winter temperatures from 0 to 20 °C. The snakes were fed freshly-killed or frozen-then-thawed mice and rats at weekly intervals in spring, summer and autumn. The brownsnakes were fed fortnightly during winter. Each snake was removed from its cage and milked of venom at fortnightly intervals for brownsnakes and weekly intervals for tigersnakes. At times, the interval between milking varied due to venom requirements. Venom was obtained as follows.

P. textilis: As described in Mirtschin et al. (1998), 100 μl pipette tips were placed on each fang without suction and the venom collected. From here the venom was forced from

the pipette tip into a small plastic vial using air from a rubber bulb placed at the end of the pipette tip. This process was repeated until no more venom could be obtained. The venom was then frozen in dry ice and freeze-dried.

N. scutatus: Initially, these snakes were forced to bite on a parafilm membrane stretched over a plastic bottle. The snake's venom glands were massaged during this time. Then, a pipette tube was placed on each fang and further venom extracted using the process described earlier for *P. textilis*.

For both species, the venom output from each individual snake was weighed and then the venom from snakes of the same species and from the same geographic location was pooled for drying. After drying, the venom vials were weighed again to determine the total dry weight of venom. This dry weight was used to calculate the percentage solids. Thus, in our data the % solids in venom reflect mean values per sample rather than individual values per snake. From these data, the dry venom yield for each snake was calculated.

Data on venom yields were collected from May 1999 to July 2001 for tigersnakes and from September 1997 to February 2002 for brownsnakes. Body lengths and head dimensions of the tigersnakes were measured in July 2001, and of the brownsnakes in February 2002. We recorded snout–vent length (henceforth, SVL), head length (from the tip of the snout to the rear of the quadrate-articular projection) and head width (across the back of the head level with the quadrate-articular projection on each side; see Fig. 1). Our analysis focused on two variables related to venom yield: the total (wet) yield, and the % solids (i.e. dry mass divided by wet mass). The product of these two variables provides an index of the total output of dried venom. To avoid pseudoreplication, we calculated mean yields per snake rather than treating successive measurements of yields from the same snake as independent values for our statistical analyses. In other analyses, we calculated mean yields per season per snake and analysed these data in a repeated-measures design with season as the repeated factor. We defined seasons as follows: summer December–February; autumn March–May; winter June–August; spring September–November. The software programs Statview 5 (SAS Institute, 1998) and SuperANOVA 1.1 (Abacus Concepts, 1991) on an Apple Macintosh G4 computer were used for all statistical analyses.

3. Results

Data were obtained for morphology and venom yields on a total of 53 brownsnakes and 36 tigersnakes. However, because morphology was measured at the end of the study, some animals provided data on venom yields but not morphology (because they were no longer part of the venom production system by the time that the morphological traits were measured). We adopted a stepwise approach to the analysis, using the framework outlined in the Introduction to this paper. Thus, we first examined whether or not the two species differed in respect to the two venom-yield traits, then (because they did differ significantly), we proceeded to look for geographic differences in yield separately within each species; and so forth.

3.1. Species differences in venom production

For this analysis, we calculated mean yields for each snake, and then compared the two species using one-factor ANOVA. Although the brownsnakes in our sample were on average longer than the tigersnakes (mean SVLs of 116.2 vs. 91.6 cm; $F_{1,83} = 59.25$, $P < 0.0001$), their venom yields were much lower. Mean yields in terms of wet mass were more than three times greater from tigersnakes than brownsnakes (means of 16 vs. 5 mg), enabling confident rejection of the null hypothesis of equal venom yields in the two species ($F_{1,83} = 67.83$, $P < 0.0001$). The % solids of the venom averaged slightly but significantly higher in brownsnakes than tigersnakes (21 vs. 20%; $F_{1,83} = 36.94$, $P < 0.0001$).

3.2. Geographic variation in venom production within species

Tigersnakes from the three localities for which we had data did not differ significantly in mean yields per snake (Fig. 2; $F_{2,32} = 1.82$, $P = 0.18$). However, the mean value for % solids was higher in the Victorian snakes (24%) than in the South Australian snakes (means of 19% in both SA populations; $F_{2,32} = 17.24$, $P < 0.0001$). Posthoc Tukey–Kramer tests showed that the Victorian tigersnakes differed significantly from both the other populations in this respect ($P < 0.05$; see Fig. 2).

For brownsnakes, Queensland animals produced an average of three times as much venom as did their South Australian conspecifics (means of 11 vs. 3 mg; $F_{1,48} = 35.76$, $P < 0.0001$). Percent solids showed the opposite trend, with mean values of 20 vs. 22%, respectively ($F_{1,48} = 15.49$, $P < 0.001$).

3.3. Effects of body length on venom production

Regression analysis revealed that mean venom yield increased with body length (SVL) for tigersnakes overall (pooling localities; $n = 35$, $r = +0.75$, $P < 0.0001$) and the same was true when we analysed data separately by locality (south-eastern South Australia $n = 23$, $r = +0.67$, $P < 0.001$; Victoria, $n = 7$, $r = +0.89$, $P < 0.01$; Lake Alexandrina, $n = 5$, $r = +0.64$, $P = 0.25$; see Fig. 3).

Given this allometry, might differences in mean body length between our samples from different populations have obscured an underlying geographic difference in venom yields relative to length? That is, did snakes from some areas

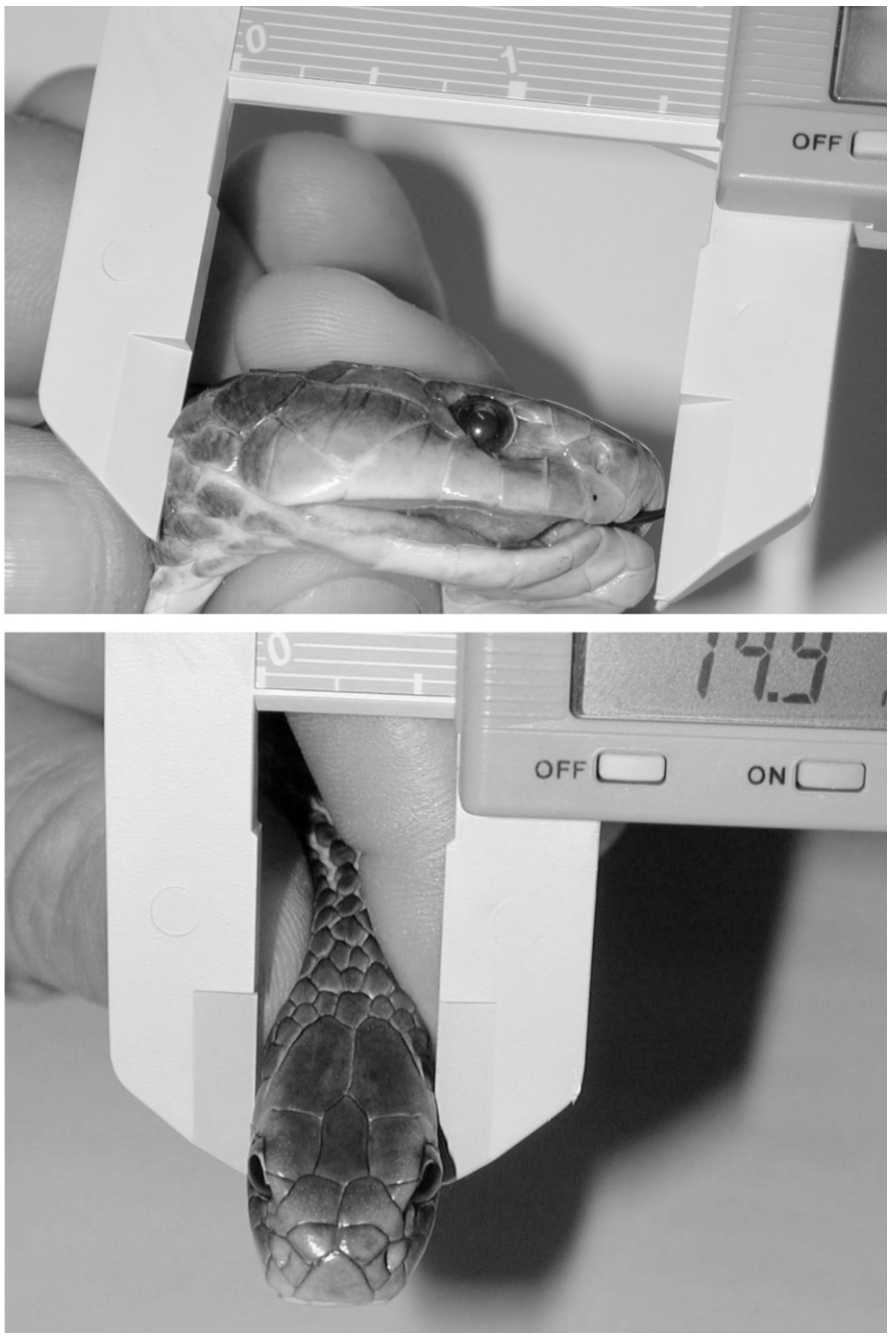

Fig. 1. Methods of measuring head length (for a tigersnake, *N. scutatus*) and head width (for a brownsnake, *P. textilis*).

produce more venom than same-sized snakes from other regions, and did we fail to detect an overall difference simply because snakes from one area were, on average, smaller? Supporting this possibility, average body lengths did differ: snakes from Victoria averaged larger (mean SVL = 97.6 cm) than those from south-eastern South Australia (92.7 cm) or Lake Alexandrina (80.0 cm; $F_{2,33} = 5.65$, $P < 0.008$; posthoc tests show that Lake Alexandrina snakes were significantly smaller on average than either of the other two populations). However, analysis of covariance with geographic origin as the factor and SVL as the covariate showed no significant geographic variation in venom yield even after the effects of SVL differences were removed from the analysis (for wet yield; slopes $F_{2,29} =$

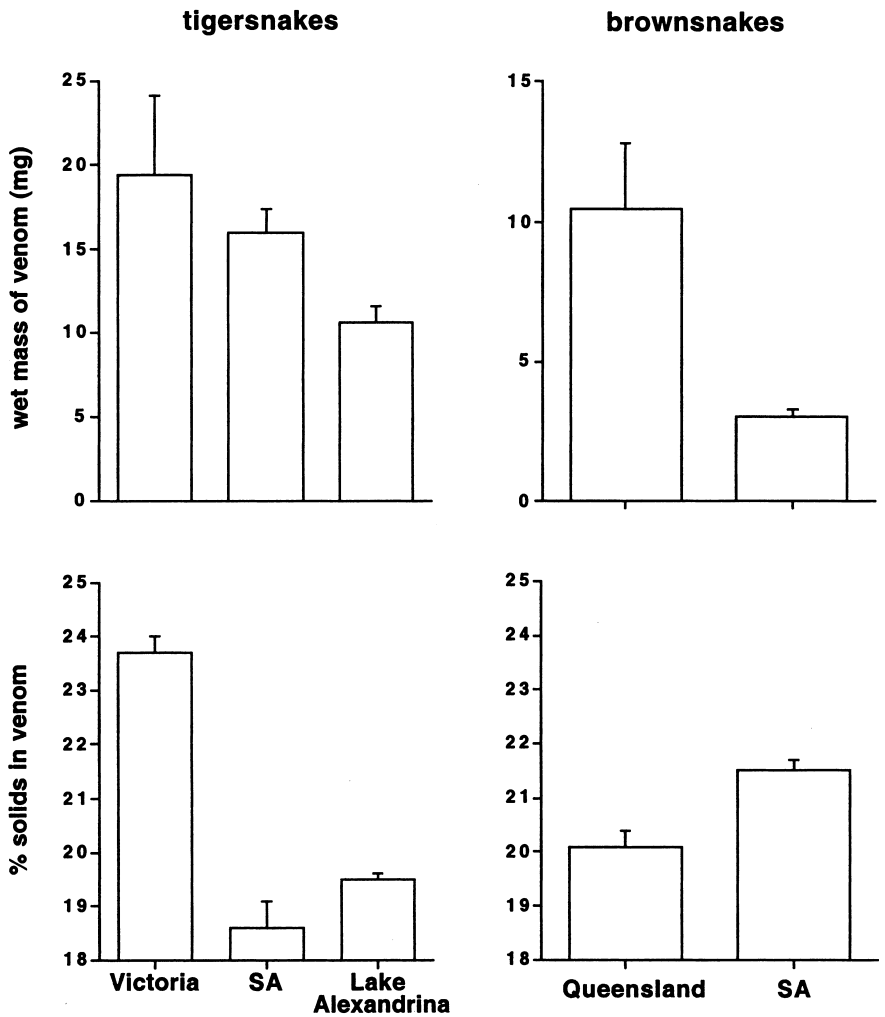

Fig. 2. Venom yields and density from tigersnakes (*N. scutatus*) and brownsnakes (*P. textilis*) from different populations. Histograms show mean values and associated standard errors. '% solids' was calculated from the dry mass of venom divided by the wet mass of venom, × 100. See text for statistical tests.

3.06, $P = 0.06$, intercepts $F_{2,31} = 0.38$, $P = 0.69$). Thus, allowing for body length differences did not reveal any geographic variation in venom yield.

In contrast to overall venom yields, % solids did not shift with the snake's body length either overall ($n = 35$, $r = +0.05$, $P = 0.78$) nor in any of the separate localities (all $P > 0.17$; see Fig. 3). Thus, allometry of this trait is unlikely to explain the significant geographic variation in % solids (above). In keeping with this inference, ANCOVA with geographic origin as the factor and SVL as the covariate showed that % solids differed significantly among populations even after this factor was removed from the analysis (slopes, $F_{2,29} = 0.17$, $P = 0.84$; intercepts, $F_{2,31} = 19.31$, $P < 0.0001$).

For brownsnakes as for tigersnakes, venom yield increased with the body length of snakes overall ($n = 50$, $r = +0.76$, $P < 0.0001$) and the same trend was evident within samples from each of the two localities (Queensland, $n = 10$, $r = +0.60$, $P = 0.06$; South Australia, $n = 40$, $r = +0.67$, $P < 0.0001$). Mean body lengths also differed between the two regions; the Queensland animals were larger than the South Australian specimens (mean SVLs of 140.9 vs. 110.1 cm; $F_{1,48} = 58.60$, $P < 0.0001$). However, we cannot determine whether the disparity in mean venom yield was simply a function of the body length difference. Venom yields increased sharply at larger body lengths, but there was insufficient body size overlap between snakes from the two locations to compare yields statistically over the same range of body lengths (Fig. 3). All we can conclude is that the Queensland animals were larger, and produced more venom.

In contrast to our data on tigersnakes, a brownsnake's body length affected the % solids in its venom (Fig. 3). Larger snakes produced less concentrated venom (overall,

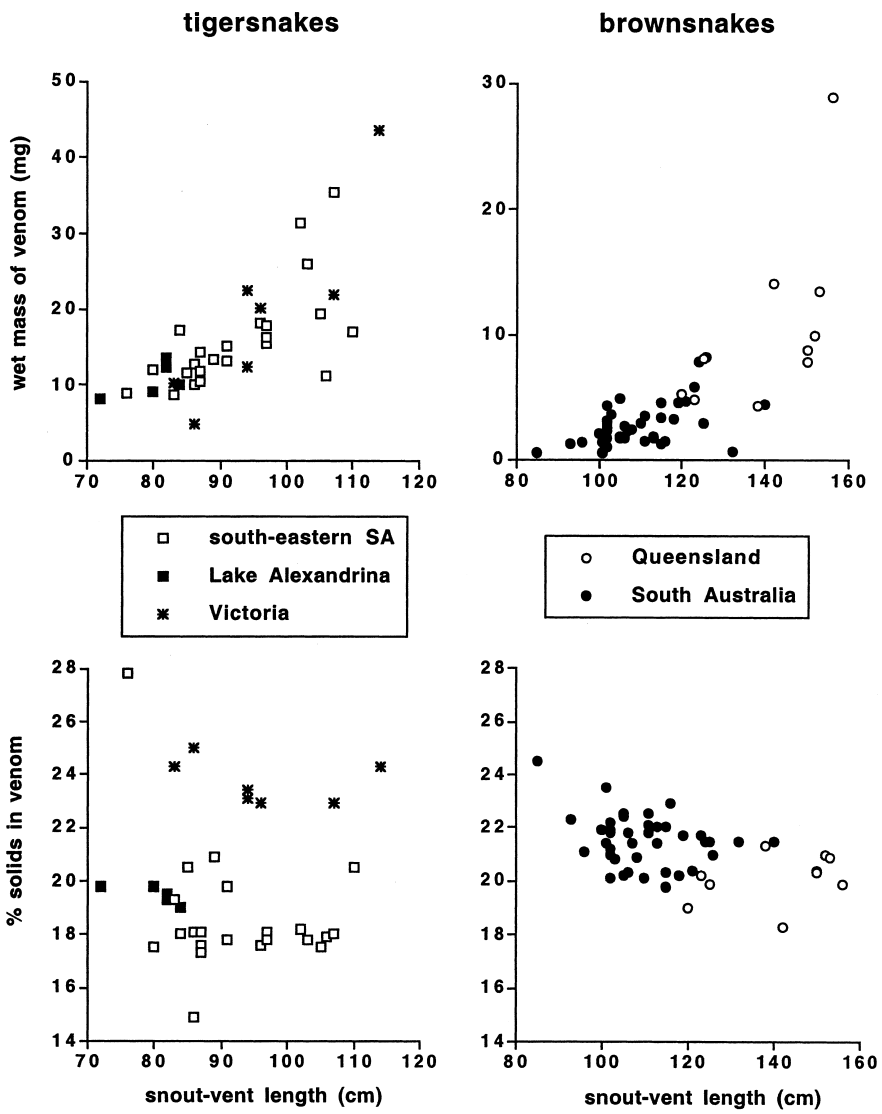

Fig. 3. Relationship between a snake's body size (snout–vent length) and the amount of venom it produced when 'milked' (upper panels), and the density of that venom (lower panels). Left-hand-side graphs show data for tigersnakes (*N. scutatus*), and right-hand-side graphs show data for brownsnakes (*P. textilis*). '% solids' was calculated as the dry mass of venom divided by the wet mass of venom, × 100. Data are shown separately for three populations of tigersnakes and two populations of brownsnakes. See text for statistical tests.

$n = 50$, $r = -0.43$, $P < 0.002$). However, this result seems to be an artefact of combining two samples with different mean body lengths; no significant trend for decreasing % solids in larger snakes was evident either in Queensland snakes ($n = 10$, $r = +0.36$, $P = 0.31$) or South Australian animals ($n = 40$, $r = -0.24$, $P = 0.14$; see Fig. 3). Thus, the overall pattern may simply reflect the fact that Queensland snakes were larger, and had less dense venom, than did the South Australian animals.

It is also of interest to ask how rapidly venom yields increase with increasing body size. If the relationship is isometric (that is, venom yield is directly proportional to mass of the snake), a regression of ln-transformed values for body length versus venom yield should display a coefficient of approximately 3.0 (Greer, 1997). The calculated coefficient for tigersnakes was exactly this value (slope = 3.00, SE = 0.45) whereas for brownsnakes, venom production increased more rapidly with increasing body length (Fig. 3; coefficient = 4.70, SE = 0.47).

3.4. Effect of relative head size on venom production

We used residual scores from the general linear regression of head length versus SVL as an index of relative head length, and head width against SVL for relative head width. A negative residual score indicates a snake with a

head smaller than expected from its SVL, whereas a positive residual score indicates an animal with a head larger than expected from its body size. Because SVL influences venom yield (above), it must be included in the analysis. Thus, we used multiple regression with SVL, relative head length and relative head width as independent variables. The wet mass of venom produced by a tigersnake increased with SVL ($t = 6.44$, $P < 0.0001$) but was not affected by the animal's relative head length ($t = 0.57$, $P = 0.57$) or width ($t = 0.50$, $P = 0.62$). Percent solids in the venom was not affected by any of the independent variables (all $P > 0.18$). The same results were obtained when data for each population were analysed separately.

For South Australian brownsnakes, in contrast, venom yield was affected by relative head length ($t = 2.51$, $P < 0.02$) as well as by SVL ($t = 8.42$, $P < 0.0001$), but not by relative head width ($t = 1.08$, $P = 0.29$). For analyses on Queensland brownsnakes, and on the overall data set, the only significant effect on venom yield was SVL (all others had $P > 0.05$). None of these variables significantly influenced venom % solids in brownsnakes (all $P > 0.05$).

3.5. Sex differences in venom production

Male tigersnakes were slightly but not significantly larger than females in our sample (means of 95.3, 89.8 cm, respectively; $F_{1,23} = 1.71$, $P = 0.20$). Sex differences were close to statistical significance for venom yield (means 20 vs. 13 mg; $F_{1,22} = 3.93$, $P = 0.06$) but were minor for % solids (20% in both; $F_{1,22} = 0.07$, $P = 0.80$). ANCOVA with sex as the factor and SVL as the covariate suggests that the apparent sex difference in yield probably reflects the minor sex dimorphism in body length; the difference in venom yield disappeared when SVL was factored out of the analysis (slopes, $F_{1,20} = 1.98$, $P = 0.17$; intercepts, $F_{1,20} = 0.85$, $P = 0.37$).

Brownsnakes showed stronger sexual dimorphism in body size, with males averaging significantly larger than females overall (121.2 vs. 105.6 cm; $F_{1,48} = 11.31$, $P < 0.002$). On average, male brownsnakes also produced about three times as much venom as did females (means = 6 vs. 2 mg; $F_{1,48} = 9.63$, $P < 0.004$), albeit at slightly lower % solids (means = 21 vs. 22%; $F_{1,48} = 4.98$, $P < 0.04$). When SVL was factored out of the analysis, the sex difference in venom yields disappeared (ANCOVA: slopes, $F_{1,46} = 3.62$, $P = 0.06$; intercepts, $F_{1,47} = 0.82$, $P = 0.37$). The same was true for % solids (when SVL was factored out of the analysis, the sex effect has slopes, $F_{1,46} = 2.12$, $P = 0.15$; intercepts, $F_{1,47} = 1.02$, $P = 0.32$). Thus, sex differences in venom yield and % solids were secondary consequences of sex difference in body size in both species.

3.6. Seasonal variation in venom production

To examine variations with season, we entered mean values for each season for each snake into a repeated-measures ANOVA with season as the repeated factor. For tigersnakes, yields were higher in summer than in spring and autumn, which in turn were higher than those in winter (Fig. 4; $F_{3,87} = 4.05$, $P < 0.001$; posthoc tests show that winter values were significantly lower than others). These seasonal patterns were seen in each of the three populations; a two-factor ANOVA with season and location as factors revealed no significant interaction between the two sources of variation ($F_{6,123} = 0.19$, $P = 0.94$). Despite significant geographic variation in % solids, there was no interaction between season and location for this parameter either ($F_{6,93} = 0.15$, $P = 0.98$), nor any significant overall effect of season on % solids ($F_{3,99} = 1.80$, $P = 0.15$).

We analysed data from brownsnakes in the same way. Repeated-measures ANOVA revealed strong seasonal variation in venom yields (Fig. 4; $F_{3,144} = 22.14$, $P < 0.0001$; posthoc tests show that spring and summer values were similar, but all other comparisons were significant at $P < 0.05$). Percent solids also varied seasonally (Fig. 4; $F_{3,144} = 45.50$, $P < 0.0001$; posthoc tests show that spring and autumn values were similar, but all other comparisons were significant at $P < 0.05$). When locality was added as a factor to these repeated-measures ANOVAs, significant interaction terms between locality and season were evident for both venom yield ($F_{3,141} = 11.91$, $P < 0.0001$) and % solids ($F_{3,141} = 4.29$, $P < 0.007$). Yields fluctuated more with season for Queensland than for South Australian brownsnakes, whereas the reverse was true for % solids.

4. Discussion

Our extensive data set (based on 1440 milkings of 36 tigersnakes, and 2734 milkings of 53 brownsnakes) revealed significant variation in both of the attributes that we studied: the wet mass of venom expelled by a snake during milking, and the % solids in that venom. Most of the factors that we investigated as potential influences did indeed generate statistically significant variation in the venom attributes, although in some cases (such as the snake's sex), the influence was an indirect one (in that case, mediated via sex differences in body size). Of the two aspects of venom yield that we examined, % solids showed much less variation than did total wet mass. Perhaps the most striking result from our analyses is the relative magnitude of different influences on venom yield. A snake's body size was by far the most important such factor, generating approximately six-fold (*Notechis*) to 30-fold (*Pseudonaja*) differences in mean yield among individual snakes (Fig. 3). In contrast, differences between the two species, and among snakes from different geographic locations within each species, both averaged approximately three-fold (Fig. 2). Seasonal

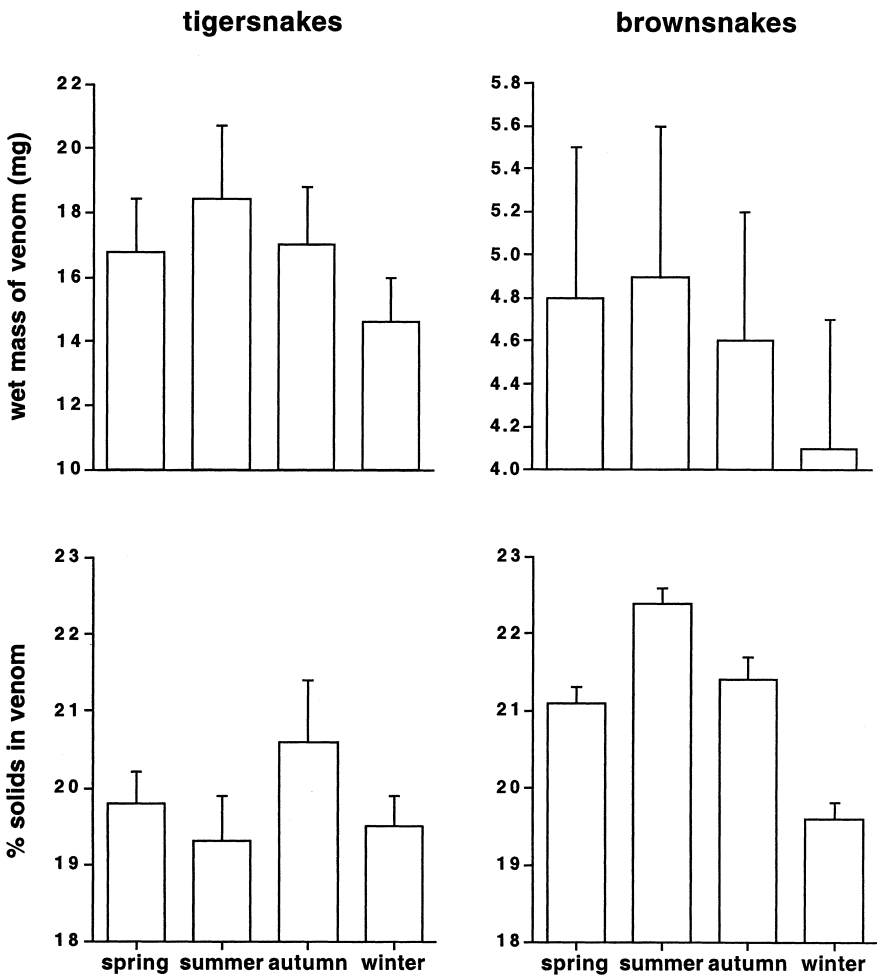

Fig. 4. Seasonal variation in venom yields and % solids in venom for tigersnakes (*N. scutatus*) and brownsnakes (*P. textilis*). Histograms show mean values and associated standard errors. '% solids' was calculated as the dry mass of venom divided by the wet mass of venom, × 100. See text for definition of seasons, and statistical tests.

variation was less marked, generally contributing a less than two-fold variation (Fig. 4).

Inevitably, there are many caveats in terms of the interpretation of our data. For example, we do not know how variation in feeding rates or in the timing of milking influences the total yield of venom that we recorded, nor how subtle seasonal variations in variables such as body condition or hydration may have influenced our results. Our data are broadly consistent with those from published studies, although comparison is difficult for most traits because there has been little quantitative research on these issues in previous work. The most straightforward comparison is for the interspecific differences. Several previous studies have documented much higher venom yields in tigersnakes than in brownsnakes, often reporting substantially greater differences than we observed. Indeed, one of the earliest studies reported that they were unable to obtain any venom by milking brownsnakes (Fairly and Splatt, 1929). We found mean values of 16 vs. 5 mg for wet mass of venom in the two species, compared to mean values of 27 vs. 3 mg in previous work (summarised by Greer, 1997; see also Fairly and Splatt, 1929; Kellaway, 1931; Freeman and Kellaway, 1934; Wiener, 1960; Worrell, 1963; Limpus, 1978; Masci et al., 1998; Sutherland and Tibballs, 2001).

One consistent feature of previous reports is that mean values for venom yields per species often vary considerably between different studies (see Greer, 1997). Our study clarifies part of the reason for these kinds of discrepancies, by identifying several sources of significant variation in venom yield. Presumably, many of the previous studies were based on animals of different mean body sizes, from different locations, milked at different times of the year. The overall differences between studies in mean values of venom yield per species will reflect a combination of all of these effects, plus others related to details of the methods

used to maintain captive snakes (Freeman and Kellaway, 1934) and to obtain venom (Sutherland and Tibballs, 2001).

Geographic variation in venom characteristics within a single wide-ranging species has been reported for several taxa of snakes. Most studies on this topic have focused on the pharmacological action of venoms, and have sometimes revealed extensive geographic variation in venom composition (Glenn and Straight, 1978; Williams et al., 1988; Daltry et al., 1997; Williams and White, 1997). Mean venom yields have also been shown to vary geographically in several snake taxa (e.g. Klauber, 1956; Mirtschin and Davis, 1992). Masci et al. (1998) and Sutherland and Tibballs (2001) reported differences between Queensland and South Australian brownsnakes, based on earlier data from some of the same snakes that we have studied. The causal factors that generate geographic variation in venom yield offer a fertile field for future investigation. Presumably, some of the spatial variation in yields reflects genetic differences among populations, whereas another part of the overall variation is directly engendered by environmental factors. For example, Fairly and Splatt (1929) recorded a threefold difference in venom yields for tigersnakes from two areas, and attributed this difference to food supply (drought had reduced prey abundance and thus the body condition of snakes in one area but not the other). Although this inference is plausible, any such geographic difference might also reflect geographic variation in body sizes (a reflection of both genetics and past environments: Madsen and Shine, 1993) as well as other factors. Because snakes in our own study were maintained in captivity for long periods prior to data collection, differences in proximate environmental cues between the localities cannot explain the persistence of geographic differences in venom yield.

An increase in venom yield with the body size of the snake is not surprising, and similar patterns are probably universal among venomous snakes (e.g. Fairly and Splatt, 1929; Marsh and Whaler, 1984; Tun and Cho, 1986; Greer, 1997; de Roodt et al., 1998). The more interesting issue is the fact that body size engenders such a high proportion of overall variation in venom yield within our data set, and is responsible for statistically significant differences in yield between sexes and among locations. Body sizes show an extremely high level of variation among snakes, not only among species, but also among populations within a species (e.g. Schwaner and Sarre, 1988; Ashton, 2002) and also among individuals within a single population (Pough, 1980). Thus, variance in body size is probably the largest single contributor to the overall phenotypic variance in venom yields among snakes overall.

The magnitude of variance in venom yields induced by body size variation will depend upon the allometric coefficient linking these two traits. This coefficient varies substantially among snakes, ranging from relatively modest increases in venom yield with large increases in body size through to very rapid increases with only small increments in size (Greer, 1997). Substantial diversity was evident even in our own study, with venom yields increasing more rapidly with body size in brownsnakes than in tigersnakes (Fig. 3). The implications for snakebite risk and commercial venom production are clear, but the underlying causes for this diversity remains obscure. Presumably, natural selection may adjust the relationship between body and venom production based on the relationship between body size and factors that determine probable venom use: for example, prey sizes, prey types and feeding frequencies. Strong positive allometry in venom yields might evolve where snakes show an ontogenetic shift from small reptilian prey to larger mammalian prey, as occurs in many populations of brownsnakes (Shine, 1989) but probably, not as often in tigersnakes (Shine, 1977, 1987). Thus, the differing patterns of venom-yield allometry in these two taxa (Fig. 3) fit well with information on ontogenetic shifts in diet. However, estimates of allometric slopes for mainland tigersnakes vary substantially in published reports; our own data are similar in this respect to those of Fairly and Splatt (1929), but Wiener (1960) data suggest a much higher allometric slope (see Greer, 1997 for calculations from these sources).

Intuition suggests that at the same overall body size (snout–vent length), a snake with a larger head would be likely to produce more venom because it would have larger venom glands. This prediction was supported for brownsnakes but not tigersnakes. Undoubtedly, the morphological relationships between overall head size and venom gland capacity are complex; for example, sex differences in head size relative to body length in snakes generally reflect disproportionate enlargement of some components of the head but not others, rather than a consistent size difference in all cranial structures (Camilleri and Shine, 1990). Like absolute body size, relative head size varies among species of snakes, among populations within species, and among individuals within populations depending on sex, age and individual phenotypes (e.g. Shine, 1991b). Relative head size may also be affected by the animal's feeding habits during early life (Bonnet et al., 2001).

Sex differences in venom yield have not attracted much previous research. Limpus (1978) and Branch (1981) concluded that sex did not affect venom production, but their sample sizes were probably too low to detect sex differences even if they occurred. de Roodt et al. (1998) showed that females produced significantly more venom than conspecific males within South American pit-vipers, but that the difference was a secondary consequence of sexual size dimorphism rather than a real difference in venom yields between males and females at the same body size. This conclusion mirrors our own. Although an early study of 12 tigersnakes concluded that males produced more venom than females, even at the same body length (Wiener, 1960), reanalysis of these data does not support the original claim. In a one-factor ANOVA on Wiener's raw data, body length influenced venom yield ($F_{1,9} = 5.17$, $P < 0.05$) but sex did not (slopes, $F_{1,8} = 0.07$, $P = 0.80$; intercepts, $F_{1,9} = 0.38$, $P = 0.55$). Greer (1997) examined the same

data, and reached similar conclusions. Nonetheless, the widespread occurrence of sex differences in body size, relative head size and feeding habits among snakes (e.g. Shine, 1991b; Daltry et al., 1997) suggests that males and females of some species will diverge in venom characteristics also.

Seasonal shifts in venom production have attracted surprisingly little attention. In an intensive study on a single brownsnake, Williams and White (1992) documented an eight-fold difference (2.7–22.2 mg) in yield from milkings over a 12-month period, but did not find any consistent seasonal effects. However, these authors reported that the composition of the venom shifted seasonally, with lower coagulant activity in summer. Because their study was not replicated across years, it is impossible to know if this was a genuine seasonal effect or simply a consequence of extended captivity. Regardless, our data show a regular and highly significant decrease in venom production in cooler months, but suggest that the absolute magnitude of this shift is relatively modest compared to other influences on venom yield (Fig. 4).

The biological significance of these multiple determinants of venom production is difficult to evaluate, given that several factors complicate the link between a snake's total venom capacity and the amount that it actually expels in any given bite. Snakes can control the amount of venom they inject, and typically use less for a feeding than for a defensive strike (Morrison et al., 1982, 1983; Young and Zahn, 2001). However, many apparent defensive strikes involve bluff, without any venom transfer (Whitaker et al., 2000; Gibbons and Dorcas, 2002). Successive strikes delivered in quick succession may see a rapid decrease in venom output (Morrison et al., 1983). We need a much clearer understanding of these flexible responses before we can interpret the biological significance of factors such as species differences in overall venom yields.

The concentration of solids within the venom is presumably also under natural selection; a higher solid content will produce a more toxic venom (all else being equal) but may be more viscous and therefore more difficult to expel at high velocity through the narrow canal within the fang, and may diffuse less rapidly through the tissues of the bitten animal. Masci et al. (1987) examined the effects of diluting venoms of *P. textilis*, *Oxyuranus scutellatus* and *O. microlepidotus* with high molecular weight enzymatic procoagulants. Venom activity was maximised at intermediate levels of enzyme concentration, so that more concentrated venom will influence the speed of dilution and thus the effectiveness of the particular venom enzyme. Interspecific differences in % solids from our own data (Fig. 2) are similar to those reported by previous studies (26.3% for *Notechis*, 21.3% for *Pseudonaja* in the summary by Greer (1997)). Intraspecific (geographic, and seasonal) shifts in % solids within the venom were unexpected findings from our study (Figs. 2 and 4). It would be interesting to examine the effect of varying degrees of hydration on venom function; in the absence of such data, the biological meaning of variation in this venom characteristic remains obscure.

In summary, our data reveal multiple influences on venom yield in Australian elapid snakes, and show that some of those influences (especially, body size) are much more important than others (especially, sex and season). It is relatively easy to derive implications concerning snakebite risk, albeit with considerable ambiguity because of our ignorance about the relationship between total venom yield versus the amount of venom actually transferred during a bite. However, forging the further link to snake biology is a much more challenging proposition. We suspect that the kinds of variation that we have documented have adaptive significance, but comprehending the evolutionary forces responsible for this diversity will require research that bridges the historically disparate disciplines of snake ecology and venom toxicology.

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