



Sexual dimorphism in snakes: different reproductive roles favour different body plans

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Because snakes have a highly simplified morphology, and many species have a wide (and broadly overlapping) range of adult body sizes within each sex, they offer an excellent opportunity to compare body composition of males and females. Evolutionary theory predicts that particular body components should be differentially enlarged in the two sexes. For example, we might expect the reproductive success of females to be enhanced by enlargement of organ systems involved in the processing and storage of energy (e.g. alimentary tract, liver, fat stores) whereas males would benefit from the enlargement of systems important for mate-searching, male–male combat and sperm competition (e.g. larger mass of skeletal muscles, tail, and kidneys). Dissection of 243 specimens of three snake species (117 *Vipera aspis*, 43 *Elaphe longissima*, 83 *Coluber viridiflavus*) broadly supported these predictions. Strong sex differences were apparent in relative sizes (masses) of all the non-gonadal body components that we weighed. For example, males consistently had more musculature (relative to body length) than did conspecific females. Dimorphism in relative muscle mass is likely to be one of the most fundamental and widespread morphological differences between males and females in the Animal Kingdom.

Keywords: body composition; sexual selection; muscle mass; snakes; sexual dimorphism

1. INTRODUCTION

'Sugar and spice and all things nice, that's what little girls are made of; slugs and snails and puppy-dogs' tails, that's what little boys are made of.' (Old English nursery rhyme)

Because reproductive success in males and females is determined in very different ways, evolutionary theory predicts that the sexes will differ not only in overall body size and shape, but in many other more subtle aspects not immediately obvious to an observer (for examples, see Darwin 1871; Andersson 1994). Nonetheless, the scientific literature on sexual selection has focused mostly on sex differences in overt characteristics such as size, shape, behaviour and the elaboration of phenotypic traits that influence reproductive success (e.g. to defeat rivals or attract mates). Less attention has been paid to features of internal anatomy, except for structures involved in mate-recognition systems (such as those involved in the production or recognition of auditory, visual and pheromonal signals: Mason & Crews 1985; Ryan *et al.* 1990). Hence, sex differences in general bodily composition (e.g. relative sizes of different organ systems) have attracted little attention from evolutionary biologists, despite the fact that analyses of domestic livestock and human beings suggest that such sex differences are widespread (for examples, see Shahin *et al.* 1986; Eissen & Fetuga 1988; Alway *et al.* 1989; Hughes *et al.* 1995).

In this paper, we use information on body composition to test simple adaptationist hypotheses on the ways in which the different reproductive roles of males and

females impose selection on different organ systems. Broadly speaking, because males produce large numbers of small and relatively 'cheap' gametes, males of most species should be under strong selection to locate and effectively court receptive females, and to maximize the probability that their sperm defeats the sperm of rival males in obtaining fertilizations (Andersson 1994). Thus, selection should favour the elaboration of skeletal musculature (which facilitates mate-searching activities and male–male combat) as well as organ systems that facilitate sperm 'competitiveness'. In contrast, females need to produce energy-rich gametes, and so may be under intense selection to obtain and process nutrients, and to store them until they are needed in reproduction. Hence, we might expect females of many species to have disproportionately large alimentary tracts, fat bodies and livers.

The dearth of interest from evolutionary biologists in testing such predictions may reflect the difficulty of interpreting sex differences in body composition in many kinds of organisms. For example, if the sexes differ significantly in overall shape or size, with little overlap between adult males and females, there is no straightforward way to compare the relative proportions of different internal components. Any sex difference in such traits might be an epiphenomenon, attributable to allometry (if absolute body sizes differ between the sexes) or to the disproportionate enlargement of particular body parts (e.g. enlarged heads or weapon systems in males). To facilitate comparison, we need to restrict attention to species with: (i) a wide and overlapping range in body sizes of adult males and females (so that sex differences can be

distinguished from allometrically forced shifts); and (ii) a relatively simple morphology without substantial sex differences in general body form. Snakes fulfil both of these criteria, and hence offer excellent opportunities to investigate the ways in which evolutionary forces have shaped male and female morphology (Shine 1993).

2. MATERIALS AND METHODS

(a) *Study species*

The asp viper (*Vipera aspis*) is a medium-sized (mean snout-vent length (SVL) = 48 cm) viviparous viperid snake. The Aesculapian snake (*Elaphe longissima*) and the European whip snake (*Coluber viridiflavus*) are both large (mean adult size approximately 90 cm (SVL)), oviparous, non-venomous colubrids (Gruber 1992). All the snakes used in our analyses were adults, based on sizes at maturation in these species (Naulleau 1992; Bonnet & Naulleau 1996*a,b*). The three taxa are broadly sympatric, but differ in important respects. The asp viper is a slow-moving terrestrial snake (Naulleau *et al.* 1996), the Aesculapian snake is a semi-arboreal active forager (Naulleau & Bonnet 1995), and the European whip snakes is a fast-moving terrestrial snake (Cioffi & Chelazzi 1991; X. Bonnet and G. Naulleau, unpublished data).

All the specimens examined in this study (117 asp vipers, 43 Aesculapian snakes, and 83 whip snakes) came from western central France (Départements des Deux-Sèvres, Vendée, Loire Atlantique and Charente Maritime). No snake was intentionally killed for this study. Almost all the autopsied snakes were killed by cats, dogs, or by the general public (including road-killed animals). Snakes were dissected shortly after death or stored at -25°C in air-tight plastic bags until dissection. For each snake, we recorded SVL(+0.5 cm) and total body mass (+0.1g). Prey items found in the stomach or in the intestine were removed before weighing, and we excluded vitellogenic and gravid females to avoid biases caused by the presence of follicles and embryos. The main components of the snakes were carefully dissected and weighed (wet masses, +0.1g): the skin, the fat bodies, the liver, the viscera (including cardio-vascular system and digestive tract, but excluding the liver), the kidneys, the

gonads, the muscle mass (including ribs and vertebrae, both of which should be negligible), the head (without skin), and tail (without skin).

Because the use of ratios introduces statistical problems, all comparisons were done using analysis of covariance (Atchley *et al.* 1976). We used SVL as the covariate in these ANCOVAs (i.e. as the measure of body size) rather than overall mass, to avoid statistical artefacts which would arise if we regressed one variable against another variable that comprised a significant fraction of the first (Atchley *et al.* 1976). We natural log-transformed all mass variables to achieve a linear relation with SVL, and restricted the ANCOVA analyses to animals within the SVL range over which both sexes were represented. Slopes for male versus female data were homogeneous in all regressions.

3. RESULTS

The three study species differed in important ways. For example, males grow substantially larger than females in the two colubrids (*Elaphe* and *Coluber*), whereas females tend to grow slightly larger than males in the asp viper (one-factor ANOVAs with sex as the factor: for *Vipera*, $F_{1,117}=6.34$, $p<0.02$; for *Coluber*, $F_{1,83}=7.78$, $p<0.01$; for *Elaphe*, $F_{1,46}=7.43$, $p<0.01$). Although the snakes used in the current study clearly represent a nonrandom sample of the general population (because a snake's vulnerability to mortality may well be affected by its body size), these results are very similar to those from much larger data sets based on our long-term mark-recapture studies (G. Naulleau *et al.*, unpublished data). Similarly, males were more heavy-bodied than females in the two colubrid species, but not in the asp viper (see table 1). Even within the latter species, however, this similarity between the sexes in overall shape (mass relative to length) masked substantial differences in the relative sizes (mass) of different components of the body (table 1).

In all three species, males had a greater muscle mass, and larger (heavier) kidneys, than did conspecific females of the same body length (figure 1; table 1). The same was true of tail mass in the two species for which we could

Table 1. *Results of one-factor ANCOVA on sex differences in relative mass of different body components of three species of snakes*

(The factor was sex, the covariate was snout-vent length, and the dependent variable was log-transformed mass of the body or some component of it. The table provides F values and associated probability (p) levels; slopes were homogeneous for all cases. Boldface font shows $p<0.05$ without correction for multiple tests; all remain at $p<0.05$ after Bonferroni corrections applied within each species. The 'larger sex' column shows whether that body component was heavier in male (M) or female (F) snakes, relative to snout-vent length.)

mass	<i>Vipera aspis</i>			<i>Elaphe longissima</i>			<i>Coluber viridiflavus</i>		
	$F_{1,116}$	p	larger sex	$F_{1,42}$	p	larger sex	$F_{1,82}$	p	larger sex
total body	2.26	0.14	—	14.45	0.0005	M	19.39	0.0001	M
fat stores	8.42	0.005	F	7.38	0.01	M	1.90	0.17	—
liver	8.79	0.004	F	0.64	0.43	—	2.28	0.14	—
viscera	35.31	0.0001	F	13.78	0.0007	F	0.10	0.92	—
skin	1.68	0.20	—	10.36	0.003	M	20.75	0.0001	M
kidney	33.13	0.0001	M	10.85	0.001	M	30.61	0.0001	M
gonads	1.43	0.24	—	3.21	0.08	—	2.29	0.13	—
muscle	7.45	0.008	M	39.34	0.0001	M	43.30	0.0001	M
tail	no data			21.10	0.0002	M	28.65	0.0001	M
head	no data			0.88	0.36	—	15.87	0.0004	M

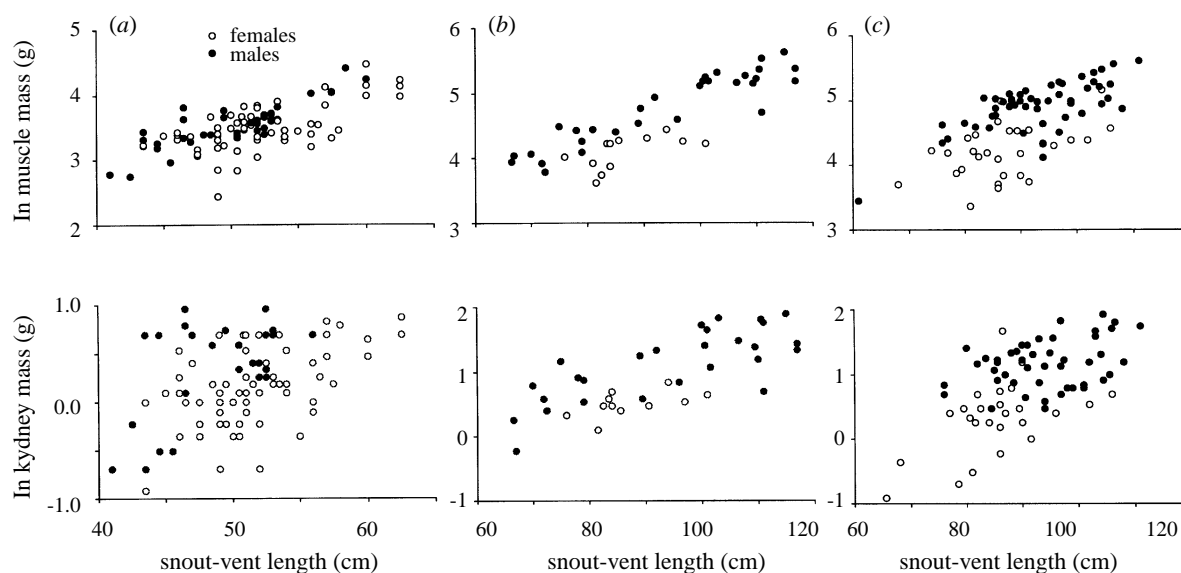


Figure 1. Sexual dimorphism in body proportions of three snake species, *Vipera aspis* (a), *Elaphe longissima* (b) and *Coluber viridiflavus* (c). At equivalent snout-vent lengths, males have a greater mass of body musculature (top graphs) and heavier kidneys (lower graphs). See the text for explanation, and table 1 for statistical analyses of these patterns.

examine this variable (table 1). Sexual dimorphism in relative masses of body components was evident for most other traits also, but was less consistent. Thus, for example, fat stores were larger (relative to svl) in males than in females in one species (*Elaphe*), but smaller in another (*Vipera*), and did not differ significantly between the sexes in a third (*Coluber*). The other main energy storage organ (the liver) tended to be larger in females than in males, but this sex difference attained statistical significance in only one species (*Vipera*). Weights of the viscera were higher in females than in males, in two of the three species (*Vipera*, *Elaphe*; table 1). More surprisingly, male *Elaphe* and *Coluber* had significantly heavier skin than did females at the same body length (table 1).

Overall, our main finding was that the sexes differ very strongly in proportional mass of various organ systems. The only exception to this pattern was, somewhat ironically, in the mass of the gonad itself (table 1). However, this null result reflects our criteria for selecting specimens for the analysis, and particularly our exclusion of vitellogenic females. If we had included the mass of vitellogenic follicles in our calculations, female gonad mass would certainly exceed that of males, at least during this phase of the reproductive cycle.

4. DISCUSSION

Previous studies on snakes have demonstrated the existence of significant sexual dimorphism in many attributes, including mean adult body size, body shape (relative length of the head, body and tail), coloration, and ecology (diets, habitat use, activity levels, and general behaviour) (for examples, see King 1989; Shine 1993). Male and female snakes of some species also differ in the relative sizes of different components of the head (Camilieri & Shine 1990). Our study extends this work to demonstrate that the body composition of male and female snakes may differ substantially, despite the strong conservatism in overall body shape within each species.

Our data on the asp viper provides perhaps the most striking case of this phenomenon. Male and female *Vipera aspis* are very similar in mean adult body size and overall body shape (mass relative to length). However, this similarity masks a series of substantial differences in body composition: at the same body length, male asp vipers have heavier musculature and kidneys, but lighter fat stores, viscera and livers, than do conspecific females (table 1).

Most, but not all, of the sex differences that we observed are consistent with predictions from simple evolutionary hypotheses. In general, males allocate a disproportionately large component of their bodies to organ systems with a plausible link to sexually selected activities such as mate-searching, male-male combat and sperm competition. In contrast, females exaggerate the relative size of organ systems related to the processing and storage of energy. However, the correlation is not perfect, as we describe below.

General body shape (mass relative to length) was similar in male versus female asp vipers, but male whip snakes and Aesculapian snakes were heavier-bodied than conspecific females (table 1). This kind of sexual dimorphism has been described in several other species of snakes, but its evolutionary significance remains obscure. In some cases it is the female that is more heavy-bodied (see, for example, Shine 1986) whereas in other cases it is the male (Boeadi *et al.* 1997). This overall dimorphism in body mass relative to length may simply reflect the sum total of all the independent effects of dimorphism in the component systems. Thus, males may be heavier than females of the same body length if the enlargement of 'male' systems (such as muscles, tails and kidneys) occurs to a greater degree than the enlargement of 'female' systems (such as the viscera and liver). Other systems may then be modified as a secondary consequence of the overall shape dimorphism. For example, total skin mass was higher in males than females of both of the species in which males were more heavy-bodied than females (table

1). Plausibly, a heavier (and thus, thicker?) body requires a larger area of skin to enclose it. However, we have no data on relative thickness of the skin, and other explanations for dimorphism in skin mass are also plausible. For example, higher activity levels or biting during male–male combat (as occurs in *Elaphe*, Carpenter 1986) might select for a thicker (tougher) body covering.

The organ systems linked to energy processing and storage tended to be larger in females than in conspecific males, as predicted. Extensive previous studies have shown the importance of energy stores for reproduction in female snakes (see, for example, Ford & Seigel 1989; Bonnet *et al.* 1994; Naulleau & Bonnet 1996). Nonetheless, at least one dimorphism that we documented is inconsistent with our predictions: we found that male *Elaphe longissima* had larger (rather than smaller) fat stores than did females (table 1). Reproducing males do not feed during the mating season in most snakes (see, for example, Madsen *et al.* 1993), including the three species used for the present study (G. Naulleau *et al.*, unpublished data). Thus, males as well as females may need to store substantial energy for reproduction (Bonnet & Naulleau 1996a). The relative magnitude of those stores in the two sexes will vary through the annual cycle, so that the time of sampling will determine which sex has the largest fat stores. In our study, male *Elaphe* were collected mainly during the mating period, because their movements are more extensive at this time (and hence, they are more likely to encounter predators and humans). Thus, our sample of male *Elaphe* may overestimate average fat stores across other seasons.

In all three species that we studied, males had larger muscle mass, kidney mass and tail mass (relative to body length) than did conspecific females (table 1). Males have longer tails than females in many snake species, probably because of the need to accommodate the hemipenes within the tailbase (see, for example, King 1989) or the advantages of longer tails in ‘tail-wrestling’ competition with rival males in mating aggregations (Madsen & Shine 1993). This sexually selected tail elongation may well explain the higher tail mass of males. Similarly, the kidneys of male snakes become hypertrophied during the reproductive cycle, and produce secretions that are combined with the semen during mating, or extruded post-insemination to form a mating plug (see, for example, Devine 1975). Kidney secretions may play an important role in sperm competition (Devine 1984). This hypertrophy is presumably responsible for the greater mass of the kidneys in males (see table 1 and figure 1).

The consistent sex difference in relative muscle mass (table 1; figure 1) is perhaps the most interesting result from our study. The functional explanation for this dimorphism is likely to involve the reliance of male reproductive behaviours (such as mate-searching and male–male combat) on muscular strength and endurance. Previous studies on snakes have demonstrated that success in these abilities is a strong determinant of male reproductive success (Madsen *et al.* 1993). Our own studies on *Vipera aspis* confirm that males in better condition (i.e. with higher mass relative to length, at the beginning of the breeding season) are more successful at obtaining mates (M. Vacher-Vallas *et al.*, unpublished data). Thus, a link

between muscular development and male reproductive success is plausible, although as yet undemonstrated.

A sexual dimorphism in relative muscle mass may be one of the most general outcomes of sexual selection, among many different kinds of animals. Although it is possible to envisage the opposite situation (e.g. advantages to greater musculature in female pythonid snakes, which use muscular contractions to warm their eggs during incubation: Ross & Marzec 1990), such cases are likely to be rare. For most kinds of animals, male reproductive success will depend more heavily on musculature (for speed, endurance or strength) than will be the case for conspecific females. Hence, we predict that dimorphism in the degree of muscular development may prove to be one of the most fundamental and consistent correlates of gender in the Animal Kingdom. Theory suggests that species with ‘reversed’ sex roles should also show a reversal in the development of musculature, providing an interesting opportunity to test the causal basis of this association.

Our interpretations have focused on ultimate rather than proximate mechanisms, and it would be possible to frame alternative explanations from the viewpoint of endocrinology (e.g. ‘testosterone stimulates muscle growth’) or phenotypic plasticity (e.g. ‘higher activity levels by males stimulate extra muscle development’). We have not taken this latter view, preferring instead to interpret the proximate mechanisms as the ways in which evolutionary optima have been attained. Nonetheless, it would be instructive to understand these proximate pathways in more detail. The considerable diversity in endocrine cycles within snakes (e.g. testosterone levels are high during mating activity in some species, but low in others, see Crews & Moore 1986) may provide an ideal opportunity to tease apart the factors contributing to the kinds of sexual dimorphism that we have documented in this paper.

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