

## SEXUAL DIMORPHISM IN TWO SPECIES OF EUROPEAN PLETHODONTID SALAMANDERS, GENUS *SPELEOMANTES*

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*Speleomantes ambrosii* and *S. strinatii* are morphologically similar but genetically well differentiated plethodontids inhabiting north-western Italy. Ten morphological characters were used to assess the amount of intraspecific sexual dimorphism in both species. On average adult females were 10% and 7% longer than males in *S. ambrosii* and *S. strinatii* respectively. ANCOVA showed that in both species there were no differences in body proportions between males and females of equal size. Multivariate analyses of size-adjusted morphological characters showed that species differed significantly in body shape, while sexes within species did not show significant overall body shape differences. The observed pattern of sexual size and shape dimorphism was similar in both species of *Speleomantes* and is discussed in relation to the reproductive biology of plethodontids.

*Key words:* *Hydromantes*, morphometrics, terrestrial salamanders

### INTRODUCTION

In salamanders sexual size dimorphism (SSD) is usually not great, with females growing slightly larger than males (Duellman & Trueb, 1986). However, in the salamander family Plethodontidae different patterns of SSD are observed (Bruce, 2000). In following the traditional taxonomy of Wake (1966), in the subfamily Desmognathinae males outgrow females in most species (Bruce, 1993; Bakkegard & Guyer, 2004), while in the tribes Hemidactyliini, Plethodontini and Bolitoglossini, all belonging to the subfamily Plethodontinae, SSD, when expressed, tends to be female-biased (Shine, 1979; Bruce, 2000). The European plethodontid genus *Speleomantes* Dubois 1984, traditionally assigned to the tribe Bolitoglossini, is no exception, as in all species females reach a larger maximum size than males (Lanza *et al.*, 1995; Lanza, 1999). However, in the North American *Hydromantes*, the sister genus to *Speleomantes*, at least one species, *H. platycephalus*, shows male-biased SSD (Adams, 1942). Moreover, recent molecular studies have shown that *Speleomantes* is allied to the plethodontine genus *Aneides* (Mueller *et al.*, 2004), wherein the pattern of SSD is variable (Staub, 1989).

To date seven species of *Speleomantes* have been described, three from south-eastern France and continental Italy and four from Sardinia, (Nascetti *et al.*, 1996; Lanza, 1999). Life histories of *Speleomantes* are characterized by complete terrestriality, egg brooding by females (Durand, 1967), and relatively long generation times. North-western Italian *S. strinatii* males become mature in their third year and females one year later [Salvidio, 1993 (at that time *S. strinatii* was considered conspecific with *S. ambrosii*)].

In *Speleomantes* the main sexually dimorphic characters are the presence in reproductive males of a well-developed chin gland, a swollen vent, and monocuspid premaxillary teeth (Greven *et al.*, 2004). According to Lanza *et al.* (1995) sexes differ in that females grow larger and males possess more projecting snouts and longer limbs. However, in their morphological analyses, Lanza *et al.* (1995) treated the two north-western Italian species, *S. ambrosii* and *S. strinatii*, together, concealing possible morphological differences between these genetically well separated taxa (Nascetti *et al.*, 1996). In addition, sexually dimorphic characters were analysed using body ratios to compare populations and species but the amount of intraspecific sexual dimorphism was not evaluated (Lanza *et al.*, 1995). Thus, the principal aims of this paper were (1) to assess the presence and amount of sexual size and shape dimorphism within each species, and (2) to determine whether any observed pattern in sexual shape dimorphism showed similar trends in the two species. We also deemed it useful to examine trends in SSD in these species with reference to the principles of sexual selection and life history theory, relative to variation in SSD, life history, and reproductive behaviour in bolitoglossine and other plethodontid salamanders.

### MATERIAL AND METHODS

#### STUDY SPECIES AND SAMPLED POPULATIONS

*Speleomantes ambrosii* and *S. strinatii* are large bolitoglossines with maximal total lengths of about 125 mm. The two species are similar in external morphology but genetically well differentiated, in that Nei's mean genetic distance, calculated over 33 genetic loci by starch gel electrophoresis, was  $D=0.33$ , range 0.26-0.44, (Nascetti *et al.*, 1996). In addition, no genetic introgression was observed between two parapatric

populations separated by a linear distance of only 1 km (Nascetti *et al.*, 1996).

In this study, 80 salamanders were measured: 40 *S. strinatii* (20 male, 20 female) collected inside two artificial cavities near Savignone (Genova), central Liguria, between 25 July and 1 September 2003, and 40 *S. ambrosii* (20 male, 20 female) captured on 5 August 2003 inside an artificial cavity near the town of La Spezia, eastern Liguria. All measurements were taken on live sexually mature salamanders: males possessing a chin gland and females measuring more than 58 mm from the snout to posterior margin of the cloaca (Salvidio, 1993; Salvidio *et al.*, 2003). After being measured all salamanders were released at capture sites.

#### MORPHOMETRIC MEASUREMENTS

Body dimensions were measured in living animals. Snout-vent length from the tip of the snout to the posterior edge of the cloaca ( $SVL_p$ ) and snout-vent length from the tip of the snout to the anterior edge of the cloaca ( $SVL_a$ ) were measured with a transparent plastic ruler to the nearest mm. Cloacal length was obtained by subtracting  $SVL_a$  from  $SVL_p$ . The following measurements were taken with vernier calipers to the nearest 0.1 mm: head length from the tip of the snout to the jaw articulation; head width as the distance between right and left jaw articulations; head depth at the level of the jaw articulations; internarial distance; orbitonarial distance from the anterior eyelid commissure to the naris; interorbital distance between anterior eyelid commissures at the proximal edge of the canthus rostralis; eye diameter; anterior body depth at the level of forelimb insertions; posterior body depth at the level of hindlimb insertions; anterior body width just anterior to the insertion of forelimbs; posterior body width just anterior to the hindlimb insertions; forearm length from the elbow to the wrist; thigh length from the leg insertion to the knee; shank length from the knee to the ankle; diameter of the brachium midway; and thigh diameter midway. Bilateral characters were measured on the right side.

To estimate measurement error, five male *S. strinatii* were selected at random and measured 10 times for all characters. Values of the coefficient of variation ( $CV = SD/mean$ ) for each character were calculated for each individual, and, following Hayek *et al.* (2001), only characters showing the highest repeatability (i.e., those displaying a mean  $CV < 0.05$ ) were selected for further analyses.

#### STATISTICAL ANALYSES

A Mann-Whitney non-parametric test was used to compare  $SVL_a$  between sexes, and analysis of covariance (ANCOVA) was used to assess differences in log-transformed variables between sexes using log- $SVL_a$  as covariate. As all transformed variables were highly correlated with body length ( $P < 0.005$  in all cases), the overall morphological variation could simply reflect body size variation. Different methods have been

proposed to obtain size-adjusted morphometric variables (see references in Adams & Beachy, 2001; Parsons *et al.*, 2003). In this paper size-adjusted variables were obtained using residuals from the regression of log-transformed variables against log- $SVL_a$  (e. g., Miles, 1994; Carr, 1996; Adams, 2000). Residuals were used in principal component analysis (PCA) and in discriminant function analysis (DFA) with cross validation. The generalised Mahalanobis distance ( $D^2$ ) was used to estimate the dissimilarity between groups (Carr, 1996; Adams, 2000). Pairwise post-hoc comparisons were performed by means of *F*-ratios using Hotelling's statistics calculated from Mahalanobis distances (Manly, 2000). As group means were tested six times, the level of significance for rejecting the null hypothesis of no difference was set to 0.008, using Bonferroni adjustment.

Although not formally presented, similar statistical conclusions were obtained using the set of scores on PC2-PC6 of transformed variables excluding those on the first principal component (PC1) that is often considered a general size factor (Somers, 1986; Adams, 2000; Adams & Beachy, 2001). In the present study, the PC1 using the untransformed variable set accounted for 54% of the total variance.

The degree of sexual dimorphism was evaluated by dividing the larger sex mean size by the smaller with the result being arbitrarily positive when females are larger and negative in the opposite case (Gibbons & Lovich, 1990). All statistics were performed using MINITAB 12.21 release.

TABLE 1. Mean coefficients of variation (CV) and ranges for morphological characters in five randomly selected male *S. strinatii*, each measured 10 times.

Measurement (mm)	Mean CV	Range	Selected for analysis
$SVL_p$	0.01	0.01-0.02	
$SVL_a$	0.02	0.01-0.02	covariate
Cloaca	0.10	0.05-0.14	
Head length	0.03	0.01-0.04	yes
Head width	0.03	0.03-0.04	yes
Head height	0.04	0.03-0.07	yes
Internarial distance	0.08	0.07-0.10	
Interorbital distance	0.05	0.04-0.06	yes
Eye length	0.07	0.06-0.08	
Orbitonarial distance	0.09	0.06-0.10	
Ant. body height	0.07	0.03-0.10	
Post. body height	0.06	0.04-0.08	
Ant. body width	0.05	0.03-0.06	yes
Post. body width	0.07	0.04-0.08	
Arm length	0.03	0.02-0.04	yes
Thigh length	0.09	0.06-0.12	
Shank length	0.04	0.03-0.04	yes
Arm diameter	0.05	0.04-0.06	yes
Thigh diameter	0.05	0.04-0.06	yes

TABLE 2. Means and standard deviations for morphological characters measured in *S. ambrosii* and *S. strinatii*. ANCOVA comparing sexes within species was based upon log-transformed selected characters using log-SVL<sub>a</sub> as a covariate.

Measurement (mm)	<i>Speleomantes ambrosii</i>			<i>Speleomantes strinatii</i>		
	Males (n=20)	Females (n=20)	ANCOVA <i>P</i>	Males (n=20)	Females (n=20)	ANCOVA <i>P</i>
SVL <sub>p</sub>	60.10±2.61	66.35±3.27		60.50±3.05	64.50±4.22	
SVL <sub>a</sub>	54.45±2.20	59.80±3.04		54.45±2.96	58.05±3.63	
Cloaca	5.65±0.88	6.55±0.95		6.05±1.91	6.45±1.05	
Head length	9.69±0.76	10.08±0.92	0.372	9.12±0.52	9.74±0.97	0.669
Head width	8.67±0.40	9.47±0.63	0.465	8.34±0.62	8.73±0.71	0.260
Head height	4.33±0.23	4.73±0.40	0.808	4.26±0.23	4.49±0.32	0.234
Internarial distance	3.30±0.21	3.40±0.26		3.56±0.31	3.46±0.41	
Interorbital distance	4.51±0.40	4.72±0.39	0.185	4.39±0.37	4.53±0.40	0.080
Eye length	3.51±0.24	3.74±0.32		3.23±0.29	3.38±0.34	
Orbitonarial distance	3.19±0.24	3.22±0.31		3.14±0.20	3.04±0.34	
Ant. body height	5.85±0.48	6.43±0.51		6.03±0.43	6.14±0.58	
Post. body height	5.61±0.28	5.84±0.52		5.83±0.37	5.82±0.60	
Ant. body width	7.42±0.75	8.30±0.73	0.162	7.90±0.70	7.22±0.46	0.080
Post. body width	6.14±0.66	6.79±0.47		6.38±0.54	6.69±0.51	
Arm length	6.71±0.33	7.04±0.38	0.601	6.71±0.39	6.82±0.51	0.424
Thigh length	5.88±0.46	5.99±0.53		6.05±0.58	6.19±0.62	
Shank length	6.48±0.35	6.93±0.44	0.510	6.67±0.41	6.87±0.46	0.210
Arm diameter	1.59±0.11	1.78±0.18	0.739	1.72±0.27	1.88±0.20	0.420
Thigh diameter	1.89±0.12	2.03±0.16	0.920	1.97±0.22	2.19±0.23	0.189

## RESULTS

Means and standard deviations of SVL<sub>a</sub> were 59.80±3.04 (range 53-66) in females and 54.45±2.20 in males (range 51-60) in *S. ambrosii*, and 58.05±3.63 in females (range 52-64) and 54.45±2.96 in males (range 49-59) in *S. strinatii*. In both species females were significantly larger than males ( $W=571.5$ ,  $P<0.001$  and  $W=514.5$ ,  $P=0.005$ , for *S. ambrosii* and *S. strinatii* respectively). The values of the SSD index were +1.10 for *S. ambrosii* and +1.07 for *S. strinatii*.

Mean CV values of morphometric measures are shown in Table 1. Overall, CVs ranged from 0.01 (SVL<sub>p</sub>) to 0.10 (cloaca length) and were normally distributed (Kolmogorov-Smirnov one-sample test  $P>0.15$ ). Apart from SVL, morphometric characters based on rigid landmarks (e.g. head bones and limbs) showed the lowest CV values; on the other hand those based on fleshy structures (e.g. nostrils and eyes) displayed moderate to high variability. In both species there were no intersexual differences in the nine characters selected for their low measurement variability (ANCOVA,  $P>0.07$  in all cases, Table 2).

The result of a PCA using nine size-adjusted variables is shown in Fig. 1. The scatterplot obtained plotting PC1 and PC2 explained 42% of the total variance and showed a certain degree of shape differentiation between species, which were partially separated on PC2. The variables having the highest contribution to PC2 were: arm diameter (0.569), head length (-0.347), forearm length (-0.373) and head width (-0.293).

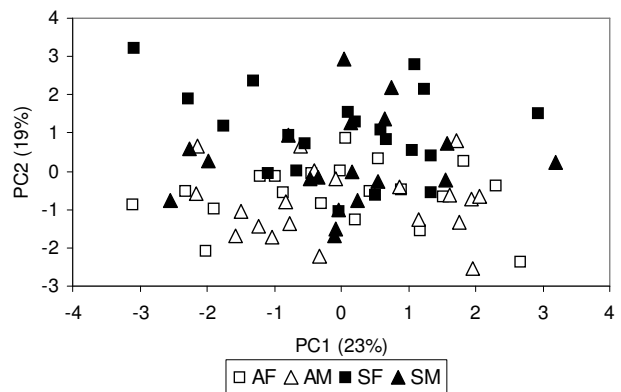


FIG. 1. Scatterplot of PC1 versus PC2 explaining 42% of the total variance. AF = *S. ambrosii* females, AM = *S. ambrosii* males, SF = *S. strinatii* females, SM = *S. strinatii* males.

On the other hand, sexes within species widely overlapped. A MANOVA using the scores of the first four PC axes (with eigenvalues > 1.0 and explaining 67% of the total variance), with sex and species as models, showed a highly significant differentiation between species (Wilks' lambda = 0.597,  $F=12.331$ ,  $df=4,73$ ,  $P<0.001$ ), while sexes and the interaction between species and sex were nonsignificant (Wilks' lambda = 0.911,  $F=1.764$ ,  $df=4,73$ ,  $P=0.145$ , and Wilks' lambda = 0.949,  $F=0.984$ ,  $df=4,73$ ,  $P=0.422$ , for sexes and interaction respectively). Discriminant function analysis with cross validation correctly classified to species 78% of individuals (62 out of 80), and 41% (33 out of 80) were correctly classified to the corresponding species and sex (Table 3). The Mahalanobis distances indicated

TABLE 3. Results of a discriminant function analysis with cross validation based on nine size-adjusted morphological characters in two species of *Speleomantes* from north-western Italy.

Classified in group	True group			
	<i>S. ambrosii</i> female	<i>S. ambrosii</i> male	<i>S. strinatii</i> female	<i>S. strinatii</i> male
<i>S. ambrosii</i> female	10	7	2	3
<i>S. ambrosii</i> male	7	10	3	4
<i>S. strinatii</i> female	1	1	7	7
<i>S. strinatii</i> male	2	2	8	6
Total <i>N</i>	20	20	20	20
<i>N</i> correct	10	10	7	6
(proportion)	(0.50)	(0.50)	(0.35)	(0.30)

that the amount of shape differentiation between sexes within species was similar ( $D^2=1.29$ ,  $D^2=0.92$ ) and did not differ significantly ( $F=1.135$ ,  $df=9,30$ ,  $P>0.008$ , and  $F=0.810$ ,  $df=9,30$ ,  $P>0.008$ , for *S. strinatii* and *S. ambrosii* respectively). However, the two species appeared relatively well-separated ( $2.65 < D^2 < 5.59$ ). The between-species  $D^2$  values differed significantly ( $F$ -ratios  $> 3.5$ ,  $df=9,30$ ;  $P<0.008$ ) with only one exception (*S. ambrosii* females - *S. strinatii* males).

#### DISCUSSION

We found that *S. ambrosii* and *S. strinatii* differed statistically in overall shape, especially in the relative dimensions of head and forelimbs. In both species adult females outgrow males by 10% (*S. ambrosii*) and 7% (*S. strinatii*), confirming earlier studies of female-biased SSD in *Speleomantes* (Lanza *et al.*, 1995). The female-biased pattern of SSD conforms to that seen in most bolitoglossines and plethodontines (Bruce, 2000), exceptions being *Thorius magnipes* (SSD index = -1.06; from Table 4 in Bruce, 2000), *Hydromantes platycephalus* (SSD index = -1.04; from Table 1 in Adams, 1942), and several species of *Aneides* (Staub, 1989). Given the sister group relationship of (1) *Speleomantes* and *Hydromantes*, and (2) *Speleomantes-Hydromantes* and *Aneides*, as proposed recently by Mueller *et al.* (2004), the variation in SSD in these taxa deserves further study. The values of SSD observed in *Speleomantes* in the present study fell in the lower range of those calculated from Bruce (2000: Tables 3 and 4) for the Bolitoglossini, in which the SSD index was on average 12% (value calculated from 27 species with both sexes represented by at least 10 individuals, including *T. magnipes*). No differences between sexes in single morphological characters were observed and multivariate shape dimorphism was slight in both *Speleomantes* species, indicating that males and females had similar overall body proportions. These results were different from those obtained in aquatic salamanders, such as *Triturus* and *Euproctus*. In the former genus, males differ from females in having longer front and hind limbs (Dandová *et al.*, 1998; Malmgren & Tholleson, 1999), while in the latter males possess wider and longer heads, stouter bodies, and longer limbs (Serra-Cobo *et al.*, 2000). In both cases, the observed shape dimorphism may be directly related to the mating

system, since in large *Triturus* species males establish and defend small territories in which they display themselves to attract females (Malmgren & Tholleson, 1999; Pough *et al.*, 2001), whereas in *Euproctus* the male engages in direct physical contact with the female, grasping her with his jaws and pushing a spermatophore toward her vent with his hindlimbs (Duellman & Trueb, 1986).

Larger size at first reproduction in females versus males is apparently a reflection of greater age at first reproduction in the former sex, estimated as 5 yr in females versus 3.5 yr in males in *S. strinatii* (Salvidio, 1993). Such a difference carries over to greater average and maximum sizes in females, as shown in *S. strinatii* by Salvidio (1993) and in both *S. strinatii* and *S. ambrosii* in the present study.

A suite of reproductive traits of female plethodontids appears to regulate adult body size. First, in female salamanders generally (Salthe, 1969), and in plethodontids specifically (Tilley, 1968; Bruce, 1969), including bolitoglossines (Houck, 1977a,b), clutch size and overall clutch dimensions tend to increase with body size, although such a relationship has not been investigated in *Speleomantes*. Secondly, growth tends to decline following maturation in female plethodontids (Marvin, 2001). And third, in most plethodontids, including some species of *Speleomantes* (Durand, 1967; Mutz, 1998), the female parent attends the egg clutch until hatching (Crump, 1995; Nussbaum, 2003). This set of traits may reflect the operation of fecundity selection, wherein larger females gain a selective advantage because they produce larger clutches and can better protect developing clutches from predators, pathogens, and desiccation. However, if growth to larger size requires a longer pre-reproductive developmental period, then, other factors being equal, growth and development to larger body size must entail a fitness cost incurred by mortality in the period of delay. Thus, trade-offs among these factors are expected to yield an optimal age/size at first reproduction as an outcome of selection in females. Alternatively, if larger size is attained by increase in growth rate, without effect on age at first reproduction, the resulting gains in fecundity may be offset by increased mortality from reductions in the allocations for repair, maintenance, and defense (Arendt, 1997). Limited data suggest that the former pathway is more

important in plethodontids (Marvin, 2001; Bruce, 2003), but data for *Speleomantes* are lacking.

If female reproductive success can be measured by fecundity and the efficacy of parental care, the comparable trait in males is success in inseminating females (Roff, 2002). In plethodontids, where courtship involves a complex tail-straddling walk, fixed at the family level (Houck & Arnold, 2003), selection for male size at first reproduction may be determined as the minimum or threshold size at which a male can successfully mate. In *Speleomantes strinatii* and *S. ambrosii*, as in most bolitoglossines, size at first reproduction in males is slightly less than that of females and this difference is achieved by a lower age at first reproduction in males.

Life-history theory predicts that smaller adult size in males versus females may be an outcome of selection in species in which competition among males for mates involves either a scramble, as in some explosive breeders, or mate searching, with minimal direct contact between males (Pough *et al.*, 2001). In contrast, selection for larger male size may occur under conditions of contest competition, involving male-male aggression and/or territorial defence of mating sites (Andersson, 1994; Shuster & Wade, 2003). In desmognathine plethodontids, where male-biased SSD is the rule in most species (Bruce, 1993, 2000; Bakkegard & Guyer, 2004), aggressive interactions have been observed between males during courtship, reflecting contest-mate competition (Houck, 1988). In *Speleomantes*, where mating seasons seem prolonged and sex ratios close to 1:1 (Salvidio, 1993; Salvidio *et al.*, 2003), there is no evidence of male aggressiveness (Salvidio, personal observation). Thus we hypothesise that female-biased SSD in *Speleomantes* (and perhaps in other bolitoglossines) may correlate with an exploitative, mate-searching mating system. Experimental studies are needed to test this hypothesis.

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