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Braincase–body size relations in European newts (*Triturus* spp., Salamandridae, Caudata): does size matter?

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A significant positive correlation between braincase size (used as a proxy for brain size) and body size was found in six European newt species, with considerable variation among the species. The observed variation in braincase size, however, could not be solely explained by variation in body size. In spite of female-biased sexual dimorphism in body size, which was especially pronounced in *Triturus alpestris*, the sexes did not differ in braincase size. We also found that *T. dobrogicus* had a much smaller braincase than would be expected considering its body size. This in addition to its different morphology and ecology sets it apart from related species of the crested newt group.

Introduction

Broadly, there are two main, not mutually exclusive, ways to address brain size issues. The first deals with the empirical notion that a larger body implies a larger brain, i.e. variation in brain size arise as a consequence of a 'scaling effect' with body size (e.g. Seyfarth & Cheney 2002). This holds especially well when comparisons are made within species, but less well when comparisons are made across species. Under the scaling assumption, the evolution of brain size can be explained as a result of selection for body size, with brain size changing as a correlated response. This hypothesis assumes a common developmental track and is characterized by a low scaling exponent value (Pagel & Harvey 1989, but *see* Mann & Towe 2003). A consequence of the 'scaling effect' would be the expectation that sexual size difference (SSD) is paralleled by sexual brain-size dimorphism as well (*see* Iwaniuk 2001).

The second hypothesis deals with consistent patterns of association between brain size, cognition and ecology that exist across animal groups (e.g. Marino 2005). Under this hypothesis, an enlarged brain has evolved as an adaptation to novel and/or altered complex environmental conditions. This hypothesis has recently gained support from the finding that large brains in birds primarily facilitate their response to changing environments by enhancing their cognitive skills (Sol *et al*. 2005).

All of the above generalities hold for the

endothermic groups (mammals and birds), while the ectotherms (fishes, amphibians and reptiles) are still underrepresented in this research area (but *see* Thireau 1975). This is the first study to evaluate brain/body size relations and their associations with morphology and ecology in European newts.

European newts (conventionally designated as the *Triturus* genus with 13 species) are the most widely distributed group of tailed amphibians within Europe and Asia (for species accounts *see* Griffiths 1996). In general, larvae and adults are pond-dwellers during the breeding season, while juveniles and non-breeding adults are terrestrial. These newts exhibit considerable variation in reproductive cycles and a higher degree of sexual dimorphism than any other tailed amphibian genus (Halliday 1977). They also have extremely complex courtship behaviour during orientation, display and spermatophore transfer phases (e.g. Halliday & Arano 1991).

We studied brain/body size relationships in the European newt at both the species and the sex levels. Population samples of six species were analyzed: the smooth newt (*T. vulgaris*), the alpine newt (*T. alpestris*) and four species of crested newts (*T. dobrogicus*, *T. cristatus*, *T. carnifex* and *T. karelinii*). The chosen newt species are different in many respects such as: phylogenetic relationships, the direction and magnitude of sexual size dimorphism, morphology, ecology and life-history traits (e.g. Macgregor *et al.* 1990, Griffiths 1996, A. Ivanović unpubl. data). Moreover, the broad interspecific range of body size of European newts is of special interest for this study. Thus, from the small *T. vulgaris*, through medium-sized *T. alpestris*, to the group of large *T.* superspecies *cristatus* newts, the adult total body length varies from 80 mm to more than 200 mm.

Material and methods

Specimens

We measured the external braincase volume in 151 individuals of six newt species: *T. vulgaris* (Velika Osječenica pond, Montenegro, n = 18), *Triturus alpestris* (Bukumirsko Lake, Montenegro, n = 21), *T. carnifex carnifex* (Velika Vala, Istria, Croatia, n = 29), *T. dobrogicus* (Ivanovo, Banat, Serbia, n = 30), *T. cristatus* (Miroč Mt., Carpathian part of Serbia, n = 31,) and *T. karelinii* (Vlasi, southern Serbia, n = 22). These samples were not taxonomically mixed as each was collected from a single breeding unit far from the zones of taxa contacts. We studied only breeding individuals with well developed secondary sexual characteristics. Namely, the genders are quite distinct due to pronounced epigamic coloring and the presence of a crest on the males. All specimens were from the Georg Džukić Batrachological Collection, Institute for Biological Research, Belgrade.

Skull preparation and estimation of external braincase volume

Newts have a dorso-ventrally flattened skull with a wide base. The elongated, ellipsoid braincase begins at the posterior margin of the nasal capsules (ethmoid plate) and its length corresponds to the length of the frontal and parietal bones that form the braincase roof (Fig. 1). The skull base and elongated parasphenoideum that form the braincase floor lie parallel to the skull roof. The medial tips of otic bones that form an arch and lie over the *foramen magnum* could be considered as the most posterior point of the brain case.

To estimate braincase volume we cleared and stained skulls for unequivocal distinction of cranial bones, sutures and cartilage (Dingerkus & Uhler 1977). Images of the skulls in the dorsal view, with frontal and parietal sutures positioned parallel to the photographic plane, were obtained with a Sony DSC-F828 digital camera (2592 × 1944 pixels resolution). In order to reduce and normalize distortion we placed skulls at the center of the optical field and photographed them alongside a 20 mm scale bar. Due to dorso-ventral flattening, the skull floor and roof bones of newts are close to being in a single horizontal plane. Using TpsDig software (http://life.bio.sunysb.edu/morph/), the same person (T.V.) digitized four two-dimensional landmarks that represent the most anterior, the most posterior and the most lateral points of the newt's brain case.

We calculated two external braincase dimensions, R1 and R2, as Euclidian distances between

the chosen landmarks (*see* Fig. 1). The third external braincase dimension (braincase height, R3) was measured using a digital caliper to the nearest 0.01 mm. Because the braincase in newts has an ellipsoid shape, we calculated its volume using the equation for the ellipsoid volume:

$$V = \frac{4}{3}\pi\left(\frac{R_1}{2}\right)\left(\frac{R_2}{2}\right)\left(\frac{R_3}{2}\right). \quad (1)$$

We used snout-vent length (SVL) measured with the digital caliper from the tip of the snout to the posterior end of the cloaca, as a proxy for the body size. The size of the external braincase was employed as a proxy for the brain size, as the brain fills the cranial cavity in most vertebrates (e.g. Emerson & Bramble 1993). Our pilot dissection study on one randomly selected female and one male specimen per species confirmed the above supposition. Although the inability to examine brain size directly is a potential limitation of this study, it should not detract from the importance of the results, as the indirect method of the cranial volume estimation seems to be appropriate for interspecies and intersex comparisons.

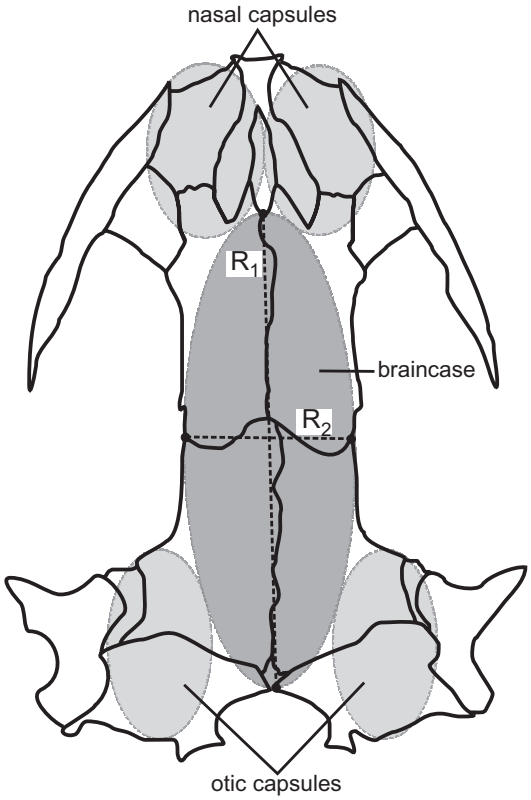

Fig. 1. Dorsal view of a newt skull and measurements used for calculation of braincase volume.

Statistical analyses

All datasets met the assumptions of normality and homogeneity of variance (Kolmogorov-Smirnov test and Levene's test). To determine differences in braincase size and body size between sexes and species, we performed two-way ANOVA for both variables separately. If either of the factors (species and sex) in the two-way ANOVA was statistically significant, we used the *t*-test or contrast analysis to test for statistical significance in pairwise comparisons employing the SAS statistical package, (SAS Inst. 1985). To estimate the strength of the linear relationship between braincase size and body size we performed standardized major axis (SMA) analysis, recommended as most appropriate for allometric analysis (Warton *et al*. 2006). The brain/body size relations among the analyzed groups (species or sexes) and comparisons of relative brain size (tests for difference in elevation of fitted slopes), were performed using the SMATR program, ver. 2.0 (http://www.bio.mq.edu.au/ecology/SMATR/).

Results

Significant variation in body size and braincase size existed within the analyzed species of European newts (Table 1 and Fig. 2). Body size increased from the smallest, *T. vulgaris*, through *T. alpestris*, *T. dobrogicus*, *T. carnifex* and *T. cristatus* to the largest, *T. karelinii*, (Table 2). Braincase size increased in the same order as the body size, with significant differences among species (Tables 1 and 2). To examine the level of sexual size-dimorphism within the analyzed samples, separate within-species analyses (*t*-test) were performed. These revealed that females were significantly larger in body size than males in *T. alpestris* and *T. carnifex* ($P < 0.005$). No statistically significant differences between the sexes in braincase size were found ($P > 0.05$ in all comparisons). The strength of the relationship between brain size and body size for each species and sex was examined using SMA analysis. We

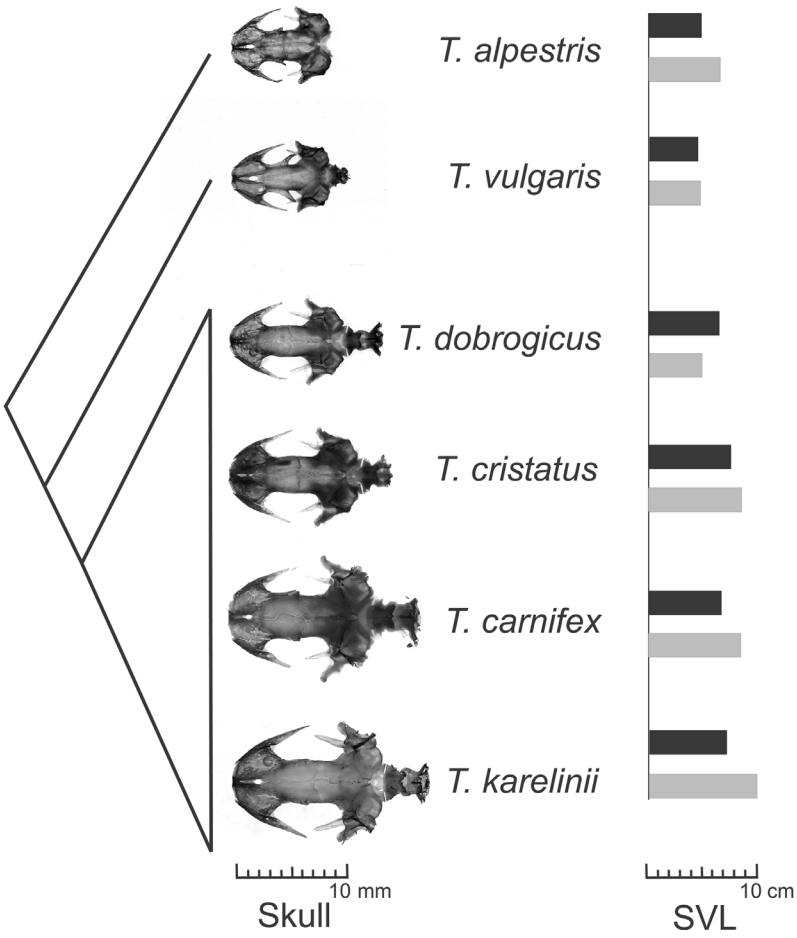

Fig. 2. Phylogenetic relationship of the smooth newt, the alpine newt and crested newts as a monophyletic group (modified from Weisrock *et al.* 2006 and Steinfartz *et al.* 2007). The photographs of the skulls were given. Black bars = body size (SVL); Gray bars = brain size/body size × 10.

observed a weak correlation between braincase and body size in *T. alpestris* males. Braincase size was unrelated to body size in *T. dobrogicus* females (Table 2).

To examine the general relationship between brain and body sizes in European newts, the SMA was performed on a pooled dataset. There was a strong, statistically significant relationship between braincase size and body size in European newts ($r^2 = 0.706$, $P < 0.0001$). The residuals, obtained from the SMA analysis, showed some consistency in the variation pattern, with males having a somewhat larger braincase volume than similarly sized females (Fig. 3). However, no significant differences in relationship between braincase size and body size were found between the sexes ($P > 0.05$ in all comparisons).

The multiple comparison of species-specific

Table 1. The variability in body size (SVL) and braincase size (external braincase volume) among European newts analyzed by two-way ANOVA with species and sex (nested within species) as factors.

Trait	Source of variation	df	SS	*F*	*P*
SVL	Species	5	16960.106	165.47	0.0001
	Sex	6	585.201	4.76	0.0002
Braincase volume	Species	5	0.046	147.14	0.0001
	Sex	6	0.001	2.19	0.0471

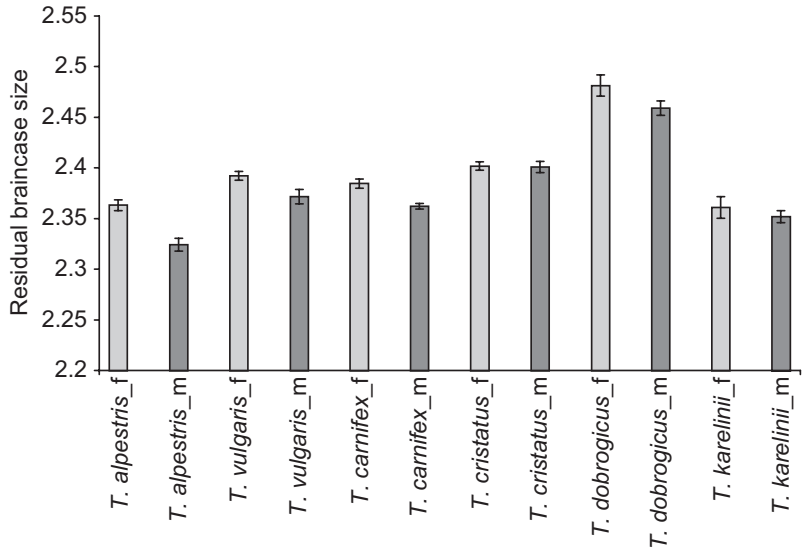

Fig. 3. The mean values (± SE) of relative braincase size (obtained as residual scores from common slope obtained by SMA analysis) for six European newt species.

slopes revealed that all analyzed species of European newts shared a common allometric slope of brain/body size relation (slope = 0.465, CI = 0.413–0.523, $P = 0.37$). To examine differences in brain size relative to body size, we tested if the species-specific slopes had a common elevation. This analysis revealed significant differences in braincase size relative to body size among the newt species (ANOVA: df = 5, $F = 89.85$, $P < 0.0001$). Pairwise comparisons showed that *T. dobrogicus* had a significantly smaller braincase than all other species ($P < 0.0001$ in all comparisons). Among *T. cristatus*, *T. carnifex* and *T. vulgaris*, no statistically significant differences in pairwise comparisons were found ($P > 0.05$ in all comparisons), while *T. alpestris* and *T. karelinii* had the largest relative brain size compared to the other species.

Discussion

European newt species analyzed thus far show a strongly expressed female-biased SSD in the alpine newt, a much less stringent female-biased SSD within crested newts, and inconsistent

Table 2. The mean values (± SE) of body size (SVL) in mm, and external braincase volume (ml) for six species of European newts. The correlation coefficients (r^2) obtained for SMA slopes fitted for each species/sex separately.

Species	Sex	*n*	SVL (mean ± SE)	Braincase volume (mean ± SE)	r^2	*P*
T. vulgaris	f	8	43.275 ± 0.907	0.019 ± 0.001	0.787	**
	m	10	41.780 ± 0.756	0.020 ± 0.001	0.557	*
T. alpestris	f	10	50.590 ± 0.875	0.032 ± 0.002	0.658	**
	m	11	44.809 ± 0.676	0.030 ± 0.001	0.272	ns
T. carnifex	f	15	68.207 ± 1.007	0.056 ± 0.003	0.701	***
	m	14	62.736 ± 1.089	0.052 ± 0.002	0.869	***
T. cristatus	f	16	75.099 ± 1.282	0.064 ± 0.003	0.737	***
	m	15	72.042 ± 1.278	0.058 ± 0.002	0.542	**
T. dobrogicus	f	15	62.685 ± 1.400	0.028 ± 0.002	0.199	ns
	m	15	62.707 ± 1.309	0.031 ± 0.001	0.475	**
T. karelinii	f	7	73.051 ± 2.817	0.075 ± 0.006	0.654	*
	m	15	68.227 ± 1.313	0.066 ± 0.002	0.469	**

ns $P > 0.05$; * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$.

results in SSD within clades of the smooth newt (Kalezić *et al*. 1992, Malmgren & Thollesson 1999, A. Ivanović unpubl. data). In the case of female-biased SSD, the males, as the smaller sex, may be expected to have smaller brains than conspecific females under the assumption that brain size is mostly determined by allometry with the body size. However, we found no sexual dimorphism in braincase size, even in *T. alpestris,* which is the most sexually dimorphic newt species. Based on these results we hypothesize that some sex-specific activities have direct or indirect effects on brain size, to the extent that male brains become equal in size to those of conspecific females. Such activities could be related to complex mating and pre-mating behaviors. Namely, during courtship males are the more active partner, from the initial extended phase of male display actions (which is a time- and space-consuming affair) up to and including the sperm transfer (e.g. Arntzen & Sparreboom 1989). In addition, territoriality, sexual interference and overt fighting, characterizes crested newts males (Zuiderwijk & Sparreboom 1986). To achieve an additional increase of brain size in males, brain size and body size (i.e. the allometric constraint) must be decoupled. This has happened in *T. alpestris* males, in which, as our results clearly show, there is no significant correlation between braincase size and body size, contrary to females from the same population.

Triturus dobrogicus has a significantly smaller brain than the other three species of this monophyletic clade of crested newts (Fig. 2). Decreasing encephalization in this species was followed with a decrease in correlation between brain size and body size, curiously only in females, while male brain size changes were firmly coupled to changes in body size. We do not have a straightforward explanation for distinctiveness of the brain/body size relation in *T. dobrogicus*, but it seems reasonable to evoke some differences in morphology and ecology that might have some indirect influence on that relation.

Among crested newts, *T. karelinii* and *T. carnifex* share basically the same phenotype: the largest body size, a stout body with a wide and long head, elongated limbs and a short interlimb distance (e.g. Kalezić *et al*. 1997, Arntzen & Wallis 1999, Arntzen 2003). This morphotype is adapted for terrestrial locomotion. *Triturus dobrogicus* individuals are smaller, have a slender and elongated trunk, a larger interlimb distance, shorter limbs and a smaller elongated head. Such a morphotype is appropriate for locomotion in aquatic habitats by sinusoidal body undulation. As expected, the life style matches the morphology. *Triturus dobrogicus* is adapted to mostly aquatic life in extensive swamps and marshes of the floodplains of the Pannonian lowland (Arntzen *et al*. 1997), spending at least six months per year in water (e.g. Jehle *et al*. 1997). In contrast, *T. carnifex* and *T. karelinii* are deme-structured newt species confined to small breeding water bodies (e.g. ponds, ditches) with small-scale geographic barriers in distribution, usually at a relatively high elevation (up to 2000 m above sea level in the south) (e.g. Griffiths 1996, Arntzen 2003). The aquatic phase of *T. carnifex*, and presumably *T. karelinii* (no data available) lasts for four months (Andreone & Giacoma 1989). The medial position of *T. cristatus* in terms of the relative brain size is in accordance with the intermedial phenotype, as well as the duration of the annual aquatic period (up to five months; Griffiths & Mylotte 1987), when compared with the two above mentioned extremes (*T. dobrogicus vs*. *T. carnifex* and *T. karelinii*).

If the level of environmental heterogeneity and brain size are positively correlated (*see* Sol *et al*. 2005 and references therein), then less-brainy newt species can be expected in the monotonic/predictable habitats. Such habitats could be relatively deep, permanent and stagnant water bodies in lowlands like the Pannonian plain, which *T. dobrogicus* exclusively inhabits. In general, a smaller brain involves much smaller energy costs for individuals and this can be an adaptive proximate factor influencing brain size in this species. In contrast, brainy newt species can be expected in much smaller, mostly temporary highland water bodies with high environmental heterogeneity, which are the habitats of *T. carnifex* and *T. karelinii*. For salamanders, as for many other vertebrates, a larger brain would be expected to allow more information to be processed, integrated and stored, as well as greater cognitive abilities (to mention the capacity for numerical discrimination; Uller *et*

al. 2003) despite the high metabolic and developmental costs. Nevertheless, without additional reliable data, associations of relative brain size with morphology and ecology cannot yet be interpreted in a causal context. Thus, ecological variables need to be translated into behavioral tasks to further our understanding of causal relationships between brain size and environment factors in these ectothermic vertebrates.

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