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Variation in skull size and shape in a newt species with male-biased sexual dimorphism

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According to Rensch's rule, sexual size dimorphism (SSD) increases with body size in groups where males are the larger sex and decreases when females are larger. The genus *Ommatotriton* represents a well-defined lineage with male-biased SSD within a group of Eurasian newts otherwise characterised by females being larger than males. In the present paper, we explore sexual dimorphism in skull size and shape for populations of the banded newt *Ommatotriton ophryticus*, applying geometric morphometrics to investigate size-dependent allometric shape variation. Sexual dimorphism in skull size was not correlated with the size of males, rejecting Rensch's rule. Sexual dimorphism in skull shape of *O. ophryticus* is entirely due to allometric, size-related shape changes between sexes.

Key words: Banded newts, geometric morphometrics, Ommatotriton ophryticus, Rensch's rule

INTRODUCTION

n amphibians, the usual pattern of sexual dimorphism (SSD) is that females are the larger sex in over 90% of anurans and 60% of salamanders; Shine, 1979; Kupfer, 2007). Eurasian newts (*sensu* Steinfartz et al., 2007) largely conform with this general pattern, although the banded newts of the genus *Ommatotriton* exhibit the opposite trend (Ivanović et al., 2008; Smith et al., 2008; Çiçek et al., 2011; Colleoni et al., 2014; for *Lissotriton vulgaris* see also Malmgren & Thollesson, 1999; Denoël et al., 2009; Ivanović & Kalezić, 2012).

According to Rensch's rule (Rensch, 1960), male-biased SSD increases with body size, whereas the opposite is the case for species and populations with female-biased SSD (Abouheif & Fairbairn, 1997; De Lisle & Rowe, 2013). In amphibians, empirical studies show that covariation between body size and SSD lead to deviations from Rensch's rule in species where females are larger than males (Ivanović et al., 2008; Liao & Chen, 2012; Liao et al., 2013; De Lisle & Rowe, 2013; Colleoni et al., 2014), whereas Rensch's rule was confirmed in amphibians with male-biased SSD (Colleoni et al., 2014). Since the size and shape of traits are necessarily related to one another, exploring these two components together provides a more complete quantification of sexual dimorphism (Berns, 2013). Allometry, the shape change associated

with size variation, could be a main component of SSD (Klingenberg, 1998). Moreover, a comparison of allometric and non-allometric components of shape variation can indicate whether changes among sexes are attributable to size alone, or influenced by other source of variation.

Pronounced sexual dimorphism in body size and significant divergence in body size among populations in Ommatotriton newts (Üzüm et al., 2014) give us an opportunity to test for Rensch's rule in a species with male-biased SSD, and to explore sexual dimorphism in shape. We applied a geometric, landmark-based approach to capture information on skull size and to explore allometric and non-allometric variation in skull shape (Mitteroecker et al., 2013). The cranial skeleton was selected because it carries important information related to the mechanics involved in feeding, and is linked to competitive, reproductive and anti-predatory behaviour (Hanken & Hall, 1993). We studied the ventral skull which consists of the upper jaw (premaxillar and maxillar bones), palate (vomeres and pterygoids), and parts involved in jaw articulation which is functionally related to biting and feeding (quadrates and squamosa). Using samples from 12 populations of Ommatotriton ophryticus we explored whether SSD follows Rensch's rule, and whether allometric scaling produces sexual dimorphism in skull shape.

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Fig. 1. Distribution map of *Ommatotriton ophryticus* and geographical positions of analysed populations. 1: Karacabey, Bursa; 2: Bahçesultan Village, Bilecik; 3: Okluca Village, Bilecik; 4: Demirbeyköy Village, Sakarya; 5: Çaycuma, Zonguldak; 6: Eflani, Karabük; 7: Tosya, Kastamonu; 8: Samsun; 9: Erbaa, Tokat; 10: Niksar, Tokat; 11: Gölköy, Ordu; 12: Rize;

MATERIAL AND METHODS

Material analysed

Ommatotriton ophryticus (Berthold 1864), the northern banded newt, ranges from the western Caucasus in southern Russia and Georgia through northwestern Armenia and northern Turkey, and west to the Bosporus Strait (Olgun et al., 2009, see Fig. 1). In total, 157 specimens from 12 populations were studied (see Table 1 for sample overview and sample sizes). All specimens were adults with well-developed secondary sexual characteristics and gonads. The skeletons were cleared with Trypsin enzyme and KOH and stained with Alizarin Red S (Dingerkus & Uhler, 1977). The cleared and stained skeletons were stored in glycerol and deposited in the collection of the Zoology Section of the Department of Biology at Adnan Menderes University, Aydın (see Online Appendix for the collection numbers).

Skulls were photographed next to a 10 mm scale bar with a digital camera. The skulls were positioned in the centre of the optical field to reduce and equalise distortion. Images of the ventral skull were taken with the palate positioned parallel to the objective of a Leica S8APO stereomicroscope connected to a computer. The photos with a scale bar were transferred to the computer through the Leica Application Suite v. 1.6.0 software. We used 13 two-dimensional landmarks on the ventral skull side which have been previously used to describe skull shape of *Ommatotriton* newts (Üzüm et al., 2014). The landmarks were digitised by the same person (N.Ü.) using TpsDig software (Rohlf, 2005) on the right side of each specimen (see Fig. 2), to avoid redundant information in symmetric structures. The left side was transposed and scored only when the right side was not accessible. Coordinates of landmarks were superimposed using Generalised Procrustes Analysis (GPA) to remove variation unrelated to shape, due to variation in position, scale and orientation (Zelditch et al., 2012).

Statistics

To explore the variation in skull size between sexes we used centroid size (CS), the geometric measure of size calculated as the square root of the summed squared distances of each landmark from the centroid of the landmark configuration (see Zelditch et al., 2012). To quantify the level of SSD, a standard index was calculated using CS values: I_{SSD} =average size of the larger sex (males)/average size of the smaller sex (females). We calculated indices of sexual dimorphism for each population separately. To estimate the standard error of

Population	Females	Males	Total
Karacabey, Bursa	5	3	8
Bahçesultan, Bilecik	5	5	10
Okluca, Bilecik	4	3	7
Demirbeyköy, Sakarya	6	4	10
Çaycuma, Zonguldak	9	5	14
Eflani, Karabük	9	6	15
Tosya, Kastamonu	7	6	13
Samsun	6	9	15
Erbaa, Tokat	10	8	18
Niksar, Tokat	6	10	16
Gölköy, Ordu	10	9	19
Rize	7	5	12
Total	84	73	157

Table 1. Overview of populations and the sample sizes inO. ophryticus

 I_{ssp} we performed a bootstrap procedure to obtain 1000 samples of m specimens (m=n) for each population and sex; $\boldsymbol{I}_{_{SSD}}$ was calculated for each of the 1000 generated datasets, using PopTools v. 2.7 to "bootstrap sample" and to calculate standard errors and confidence intervals. Differences in SSD were evaluated using analyses of variance for each population in SAS (SAS Institute Inc., Cary, NC, v. 9.1.3). In order to test Rensch's rule, CS was logarithmically transformed. We performed major axis regression to test whether a bivariate plot of log CS of females versus log CS of males has a slope significantly different from 1.0 (isometry). Female size was plotted on the x - axis and a slope significantly diverging from isometry provided evidence for Rensch's rule (Fairbairn, 1997). This analysis was performed using SMATR v. 2.0 (Falster et al., 2006).

To explore divergence in skull shape between sexes, we calculated an index of shape dimorphism (I_{sshp}) as the Procrustes distances between mean shapes of females and males. The statistical significance of Procrustes distance was obtained by a permutation test with 1000 iterations. To estimate the allometric component of shape variation we performed multivariate regression of Procrustes coordinates on CS within each sex. The statistical significance of regressions was estimated by a permutation test with 1000 iterations against the null hypothesis of independence between shape and CS. Residuals from multivariate regression were used to calculate Procrustes distances between females and males for allometry corrected shape variables. Analyses and visualisations of divergence in skull shape between females and males were done using MorphoJ software (Klingenberg, 2011).

RESULTS

Differences in skull size

The mean CS values for the ventral skull are shown in Online Appendix Table 1. Males were significantly larger than females in 10 of the 12 populations (Table 2), with significant variation in skull size between populations (factorial ANOVA, CS as dependent variable, population as a factor: F=54,43; df=11; p<0.001). The relationship between the increase in size of males and SSD was not consistent with Rensch's rule, as the degree of sexual dimorphism was constant irrespective of male and female size (Table 2). The obtained slope of the major axes regression of male skull size on female skull size was not significantly different from 1.0 (R^2 =0.939, Slope=1.016, Intercept 0.017, Cl=-0.32 - 0.35, p=0.847).

Ventral skull shape significantly differed between females and males (I_{sshp}=0.022, p<0.001). The multivariate regression of Procrustes coordinates on CS showed that 4.2% of the observed variance was explained by size (p<0.001). The shape changes of the ventral skull with size are presented in Fig. 3. The allometric shape changes were related to the changes in the position of pterygoids and quadrates (landmarks 3 and 4) and maxillae (landmark 8). Larger individuals have a shorter otico-occipital region and wider and a more posteriorly positioned quadrates. After size correction, no differences in ventral skull shape between females and males were found (Procrustes distance=0.011; p=0.09). The divergence in skull shape between sexes with and without considering allometry is presented in Fig. 4. Major changes were related to the position of pterygoids (described by landmarks 2, 3, 4, 8) and the shape of the otico-occipital region (landmarks 1, 2, 5).



Fig. 2. Ventral side of the *Ommatotriton ophryticus* skull and landmarks scored : 1, tip of occipital condyle; 2, posterior pterygoid; 3, anterior tip of pterygoid; 4, most lateral point of the quadrate; 5, vomeral teeth row– posterior ; 6, vomeral teeth row– anterior; 7, most anterior point of the vomer; 8, tip of the maxilla; 9, most posterior point of the premaxilla;10, anterior end of suture between the premaxilla and maxilla; 11, posterior margin of the choana; 12, snout tip; and 13, most posterior pterygoid.

Table 2. Indices of size (Issd) dimorphism for the ventral skull of *O. ophryticus* populations. A critical level of significance is 0.05.

Population	ISSD ± SE	p
Karacabey, Bursa	1.06 ± 0.0006	ns
Bahçesultan, Bilecik	1.13 ± 0.0006	<0.001
Okluca, Bilecik	1.08 ± 0.0012	ns
Demirbeyköy, Sakarya	1.11 ± 0.0011	0.015
Çaycuma, Zonguldak	1.13 ± 0.0005	<0.001
Eflani, Karabük	1.14 ± 0.0005	<0.001
Tosya, Kastamonu	1.13 ± 0.0009	<0.001
Samsun	1.11 ± 0.0006	<0.001
Erbaa, Tokat	1.11 ± 0.0006	<0.001
Niksar, Tokat	1.09 ± 0.0021	0.021
Gölköy, Ordu	1.08 ± 0.0004	<0.001
Rize	1.17 ± 0.0013	<0.001
Species level	1.11 ± 0.0021	<0.001

DISCUSSION

Rensch's rule, the correlation between changes in the body size and the extent of sexual dimorphism, has been confirmed for many taxa (Abouheif & Fairbairn, 1997; Fairbairn et al., 2007). It has been hypothesised that such a relationship is driven by sexual selection and a relatively high genetic correlation between sexes, leading to male body size to vary more over evolutionary time than the body size of females (Fairbairn, 1997; Blanckenhorn et al., 2007; Dale et al., 2007; Colleoni et al. 2014). Variation in body size and SSD is also expected among populations within species, which can be explained by populationspecific sexual selection or differential responses to abiotic and biotic environmental factors (Fairbairn, 2005; Blanckenhorn et al., 2006). Although studies of Rensch's rule at intraspecific level obtained mixed results (Fairbairn, 1997; Young, 2005; Frynta et al., 2012) it is expected for species with male-biased SSD (Blanckenhorn et al., 2007). Salamanders have indeterminate growth, and show considerable variation in body size among populations. Despite substantial variation in skull size O. ophryticus, we found that the magnitude of SSD remained constant. For example, De Lisle & Rowe (2013) suggested selection on females to be a driver of both amphibian allometry and SSD. The sample size of our study however precluded high statistical power of the analyses we performed.

Previous studies of sexual dimorphism in skull shape reported that dimorphism tends to be proportional to differences in size in the presence of non-allometric differences in skull shape between sexes (Bruner et al. 2005; Cardini & Elton, 2008; Ljubisavljević et al., 2010). Subtle divergences in skull shape between sexes were found in other Eurasian newt species from the genus *Ichthyosaura, Lissotriton,* and *Triturus* regardless of SSD patterns (e.g., Ivanović & Kalezić, 2012). Differences in size and shape of cranial parts may have important biomechanical and ecological implications such as divergence in feeding performances or different prey preferences between sexes (Herrell et al., 2001, 2007).



Fig. 3. Allometric shape changes within the species for the ventral skull. The wireframe graphs illustrate the shape changes from specimens with the smallest CS (lite gray wireframe) to specimens with the largest CS (black wireframe).

Allometry included Allometry excluded

Fig. 4. The shape changes of ventral skull between females and males at the species level with allometry included and with allometry corrected data. The wireframe graphs illustrate the shape changes from females (lite gray wireframe) to males (black wireframe).

We found that the divergence in the shape of ventral cranium in *O. ophryticus* is a result of differences in size between sexes, and corresponding size-related allometric changes in shape. Therefore, sexual dimorphism in skull size and shape in *O. ophryticus* can be explained by selection for larger size in males alone. One of the specific characteristics of *Ommatotriton* newts is that the males are more territorial than other European newts (Raxworthy, 1989; Bogaerts et al., 2013). Therefore, the explanation of sexual dimorphism in the ventral skull in *Ommatotriton* is likely related to courtship and fighting behaviour between males, especially biting.

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