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Sexual dimorphism and age of Mediterranean salamanders

Sandy Reinhard[a,1], Sandra Renner[a,1], Alexander Kupfer[b,c,*]

[a] Institute of Systematic Zoology and Evolutionary Biology with Phyletic Museum, Friedrich Schiller-University Jena, Erbertstraße 1, D-07743 Jena, Germany
[b] Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Straße 24-25, D-14476 Potsdam, Germany
[c] Department of Zoology, State Museum of Natural History Stuttgart, Rosenstein 1, D-70191 Stuttgart, Germany

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ABSTRACT

We analysed sexual size dimorphism (SSD) for two Mediterranean species of the “true” salamander clade possessing distinct life histories (*Salamandra algira* and *Mertensiella caucasica*) and equilibrated the morphometric approach to individual age by using skeletochronology. For species that have a short breeding season and live at high altitudes, such as Mediterranean amphibians, the fecundity advantage hypothesis predicts female-biased SSD to maximise reproductive success. Our results showed no SSD in either species; however, morphometric data indicated a male-biased dimorphism in limb (arm and leg) dimensions in both species when compared to body size. Limb dimorphisms are likely related to the particular mating system, which involves an amplexus during spermatophore transfer. Arm length appeared sexually dimorphic during ontogeny both in viviparous *S. algira* and oviparous *M. caucasica*. A review on SSD indicated monomorphy of body size as a common lineage-specific pattern among the “true” salamander clade, but also the common presence of other traits such as sexually dimorphic limb proportions.

1. Introduction

Sexual size dimorphism (SSD) describes differences in body size between males and females. In his seminal work on sexual selection, Darwin (1871) recognised this phenomenon as common within the animal kingdom and summarised possible proximate causes of SSD. SSD can be considered an adaptation to distinct reproductive roles of males and females, resulting in a difference in adult body size between the two sexes (Fairbairn, 1997). As a general pattern, male-biased dimorphism is likely to occur in endothermic vertebrates (Fairbairn, 1997; Isaac, 2005). However, among poikilothermic animals, such as amphibians, male size rarely exceeds female size, e.g., 90% of anurans and 61% of salamanders show a female-biased dimorphism (Shine, 1979; Kupfer, 2007). Female size is often positively correlated with fecundity, hence large females are able to produce larger clutches and in some cases also larger eggs or offspring (Shine, 1988; Blanckenhorn, 2005). Additionally, sexual dimorphism of traits not directly linked to reproduction can be caused by ecological differences between the sexes (Shine, 1988); e.g., the intersexual variation of adult head size is caused by ecological niche divergence (e.g., Fairbairn, 2007). Although amphibians are renowned for highly variable life histories (summarised in Wells, 2007), our understanding of SSD patterns in amphibians is still incomplete in comparison with other vertebrates (see Fairbairn et al., 2007; Kupfer, 2007).

The “true” salamanders and newts (Urodela: Salamandridae) are highly suitable for studying intra- and interspecific patterns of sexual size dimorphism, as 56% of the species exhibit female-biased and 15% male-biased size dimorphism (Kupfer, 2007), and overall they show variable reproductive modes including oviparity and viviparity combined with variable morphologies and body shapes. Sexual dimorphism is common among the Salamandridae. In most cases, the dimorphisms are discrete and have been demonstrated qualitatively, such as the presence of male secondary sexual characteristics (Sever and Staub, 2011) or differences in head size (e.g., Serra-Cobo et al., 2000) or tail length (e.g., Inger, 1947; Malmgren and Thollesson, 1999). However, few studies have quantitatively assessed sexual dimorphism of the overall bauplan of salamandrids using standard morphometrics and related their findings to age. Age should be included into the analyses to fully account for effects on SSD patterns but also to follow dimorphic traits during ontogeny (see also Monnet and Cherry, 2002; Kupfer, 2007).

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* Corresponding author at: Department of Zoology, State Museum of Natural History, Rosenstein 1, D-70191 Stuttgart, Germany. Tel.: +49 (0) 711 8936 114; fax: +49 (0) 711 8936 100.
E-mail addresses: alexander.kupfer@smns-bw.de, axkupfer@uni-potsdam.de (A. Kupfer).

[1] These authors contributed equally to this work.

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Table 1
Morphometric body and head characters.

Character	Definition
Body measures	
OAL	Overall length
SVL	Snout–vent length from the tip of the snout to the posterior edge of the cloaca
TL	Tail length from the posterior edge of the cloaca to the tip of the tail
TH	Tail height
UAL	Upper arm length from the posterior margin of the front leg (axilla) to the angle of the elbow
LAL	Lower arm length from the angle of the elbow to the wrist
LAL2	Lower arm length measured until the tip of the longest finger
TAL	Total arm length from the axilla to the tip of the longest finger
ULL	Upper leg length from the anterior margin of the hind leg (groin) to the angle of the knee
LLL	Lower leg length from the angle of the knee to the ankle
LLL2	Lower leg length measured until the tip of the longest toe
TLL	Total leg length from the groin to the tip of the longest toe
CL	Cloacal length
CW	Cloacal width
CHW	Chest width from axilla to axilla
BWM	Body width at mid-body
GG	Distance from groin to groin
DEX	Distance of extremities from axilla to groin
UAD	Upper arm diameter
LAD	Lower arm diameter
ULD	Upper leg diameter
LLD	Lower leg diameter
Head measures	
HL	Head length
HW	Head width at the angle of the jaw
ED	Diameter of the eye
ON	Orbit–naris distance from the anterior edge of the eye to the nostril
IO	Interorbital distance from eye to eye (measured from the centre of the eye)
IN	Internarial distance from nostril to nostril
ES	Eye–snout distance from the anterior edge of the eye to the tip of the snout
IC	Intercanthal distance from the anterior edge of the eye to the other

For the present study we used two species possessing distinctly different life histories, i.e., the heavy-bodied *Salamandra algira* and the slender elongated *Mertensiella caucasica*, both inhabiting Mediterranean regions, to test SSD in the "true" salamander clade. We also equilibrated the morphometric approach to individual age by using skeletochronology. The North African fire salamander, *S. algira*, is a terrestrial salamander living in habitats located in Mediterranean regions of Morocco, Algeria and Spain, i.e., Ceuta (see Beukema et al., 2013). *S. algira* is viviparous and gives birth to aquatic larvae or fully developed juveniles (Donaire-Barroso and Bogaerts, 2001). The Caucasian salamander *M. caucasica* occurs at high altitudes in western Georgia and adjacent parts of north-east Turkey (Griffiths, 1996; Franzen, 1999). It is oviparous, reproduces in streams and its life cycle includes an aquatic, lotic larval stage (Klewen, 1991).

2. Materials and methods

2.1. Morphometric analysis

Thirty morphometric characters were selected for the analysis of sexual dimorphism (defined and summarised in Table 1). Arm and leg diameters were measured vertically to the body axis. All measures were taken by one author using digital callipers (Rok International Industry, Ltd., Shenzhen, PR China) to the nearest 0.01 mm.

Altogether, 42 males and 17 females of *S. algira* originating from near Taza (Morocco) were used for analysis (see Table S1 in the supplementary online Appendix for specimen numbers). Due to the colouration and the sampling locality we referred to the animals as *S. algira splendens* (see Beukema et al., 2013). The specimens were sexed via externally visible sexual characters (e.g., swollen cloaca) and also by inspection of the gonads employing a small ventrolateral incision. The *M. caucasica* sample included 26 females and 22 males from the Zigana pass (Trabzon province, north-eastern Turkey, see Table S1 for specimen numbers). All specimens were sexed through externally visible characters, such as the swollen cloaca or prominent protuberances on the dorsal surface of the male tail base (Sever et al., 1997).

The sexual dimorphism index (SDI; after Gibbons and Lovich, 1990) was calculated by dividing the mean snout–vent length (SVL) of the larger sex by the mean SVL of the smaller sex and subtracting 1 ($\mathrm{SVL_{large}/SVL_{small}} - 1$) to obtain a rough impression of sexual size dimorphism. All characters measured were tested for normality using the D'Agostino and Pearson omnibus normality test. Univariate *t*-tests (two-sided at $\alpha \leq 0.05$) were used to test for dimorphism between sexes. In addition, SSD was assessed by an analysis of covariance (ANCOVA) using SVL as co-variable to adjust the characters to body size. Correlation tests of body measures and age were carried out using the Spearman correlation analysis. All statistical tests were conducted using Microsoft Excel 2007, GraphPad Prism 4.0c and Minitab 10.51 for Macintosh computers. Specimens were photographed using a Nikon D50 digital SLR or a Kodak ZD710 digital camera.

2.2. Histology and skeletochronology

Ageing analysis in vertebrates, including amphibians, is traditionally carried out using skeletochronology (for a review see Smirina, 1994). Skeletochronology visualises periodical skeletal growth using cross-sections of bone material. "Growth marks" (bright and broad bone material due to active osteogenesis) and "lines of arrested growth" (LAGs; distinctive thin bone layers caused by stagnation of growth) are used to estimate individual age (e.g., Alcobendas and Castanet, 2000).

To assess the age of the salamanders, the longest toe of the hind leg was clipped off from *S. algira* and *M. caucasica* specimens. The phalanges were embedded in paraffin wax. Cross-sections (thickness: 7 μm) were produced using a microtome (HM 360; Microm, Walldorf, Germany) with a stereo microscope (Zeiss Stemi 1000; Carl Zeiss Microscopy, Jena, Germany) and stained using cresyl violet (cf. Scholz et al., 2010). LAGs on at least 20 serial cross-sections per specimen were counted three times by one author using an optical microscope (Zeiss Axioskop; Carl Zeiss Microscopy, Jena, Germany) with a mounted Pixelink PL-B622CF camera (PixeLINK, Ottawa, Canada). The histological sections are stored at the State Museum of Natural History Stuttgart (SMNS, Stuttgart, Germany).

All age data were tested for normality using the D'Agostino and Pearson omnibus normality test. To allow comparisons between distinct age groups, specimens were sorted into four age groups (1+ to 5; 5+ to 10; 10+ to 15; 15+). SVL as well as total arm length (TAL) were compared within these groups (*t*-test, two-sided, $\alpha \leq 0.05$) and the respective SDI was also calculated for selected body characters within the age classes.

3. Results

3.1. Patterns of sexual size dimorphism

The sexes of *S. algira* did not differ in general body size (see Fig. 1a). The SDI was 0.0385 (implying only a weak tendency towards a female-biased dimorphism). However, *t*-tests showed significant intersexual differences in arm length and diameter and